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Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures

Risk Assessment Forum Technical Panel

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LIST OF ABBREVIATIONS

ACGIH	American Conference of Government Industrial Hygienists
AHH	Aryl Hydrocarbon Hydroxylase
ATSDR	Agency for Toxic Substances and Disease Registry
B[a]P	Benzo(a)pyrene
BINWOE	Binary Weight-of-Evidence
BMD	Benchmark Dose
CRAVE	Carcinogen Risk Assessment Verification Endeavor
ED_x	Effective Dose in x Percent of Test Animals
GSH	Glutathione
HI	Hazard Index
HQ	Hazard Quotient
IRIS	Integrated Risk Information System
LD_x	Lethal Dose in x Percent of Test Animals
LOAEL	Lowest-Observed-Adverse-Effect Level
MFO	Mixed Function Oxidase
MOAEL	Minimum-Observed-Adverse-Effect Level
MOE	Margins of Exposure
MT	Metallothionein
NAS	National Academy of Sciences
NOAEL	No-Observed-Adverse-Effect Level
NRC	National Research Council

LIST OF ABBREVIATIONS (continued)

OSHA	Occupational Safety and Health Administration
PAH	Polycyclic Aromatic Hydrocarbon
PBPK	Physiologically Based Pharmacokinetics
PBPK/PD	Physiologically Based Pharmacokinetics and Pharmacodynamics
PCB	Polychlorinated Biphenyl
POM	Polycyclic Organic Material
RfC	Reference Concentration
RfD	Reference Dose
RPF	Relative Potency Factor
TEF	Toxicity Equivalence Factor
TEQ	2,3,7,8-TCDD Toxicity Equivalents
TOC	Total Organic Carbon
TTC	Toxicity-Specific Concentration
TTD	Target Organ Toxicity Dose
UF	Uncertainty Factor
WHO	World Health Organization
WOE	Weight of Evidence

PREFACE

The U.S. EPA's Risk Assessment Forum (Forum) is publishing the *Supplemental Guidance for Conducting Health Risk Assessment of Chemical Mixtures* as a supplement to the EPA's *Guidelines for the Health Risk Assessment of Chemical Mixtures (Guidelines)*(U.S. EPA, 1986) (Appendix A). The 1986 Guidelines represent the Agency's science policy and are a procedural guide for evaluating data on the health effects from exposures to chemical mixtures. The principles and concepts put forth in the Guidelines remain in effect. However, where the Guidelines describe broad principles and include few specific procedures, the present guidance is a supplement that is intended to provide more detail on these principles and procedures.

To address concerns over health risks from multichemical exposures, the U.S. Environmental Protection Agency published the *Guidelines for the Health Risk Assessment of Chemical Mixtures* in 1986 (U.S. EPA, 1986) (Appendix A). The Guidelines describe broad concepts related to mixture exposure and toxicity and include few specific procedures. In 1989 EPA published guidance for the Superfund program on hazardous waste that gave practical steps for conducting a mixtures risk assessment (U.S. EPA, 1989a). Also in 1989, EPA published the revised document on the use of Toxicity Equivalence Factors for characterizing health risks of the class of chemicals including the dibenzo-dioxins and dibenzofurans (U.S. EPA, 1989b). In 1990, EPA published a Technical Support Document to provide more detailed information on toxicity of whole mixtures and on toxicologic interactions (e.g., synergism) between chemicals in a binary (two-chemical) mixture (U.S. EPA, 1990). The concept of toxicologic similarity was also discussed. The Environmental Criteria and Assessment Office (now the National Center for Environmental Assessment) followed this with the production of a *Technical Support Document on Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1990b).

This supplementary guidance document is a result of several influences. Because the science of environmental risk assessment has continued to evolve and EPA has learned from an array of experiences, the Agency charged the Risk Assessment Forum with developing guidance on challenging issues such as cumulative risk assessment. Part of the Forum's response to this charge was to establish a Technical Panel to ensure that the advances in the area of chemical mixtures health risk assessment are reflected in Agency-wide guidance materials. Through the evaluation of waste sites for mixtures risks it has become apparent that the exposure scenarios for these sites are extremely diverse. Moreover, the quality and quantity of pertinent information available for risk assessment has varied considerably for different mixtures. Other Agency and external initiatives have influenced the development of the chemical mixtures supplementary guidance:

- The National Academy of Sciences has issued a recommendation to move away from single-chemical assessments. (NRC, 1994)

- In 1997, EPA's Science Policy Council issued a policy statement on cumulative risk assessment. This policy addressed the first step in the overall assessment process (i.e., problem formulation) (U.S. EPA, 1997a).

- Siting activities have raised the issue of multiple chemical exposures. Parties are concerned not only about what risks are associated with releases from a particular facility, but also the potential combined effects of exposures from other sources in the area.

- EPA's research strategy for 2000 and beyond emphasizes research on chemical mixtures.

When the 1986 Guidelines were published, the Agency recognized that the Guidelines would need to be updated as the science of chemical mixture assessment evolved. Research efforts were undertaken immediately and by 1988 Agency offices were discussing revision topics. By 1989, under the auspices of the Risk Assessment Forum, efforts were underway to revise the Guidelines. Updates to the Guidelines were reviewed in a June 1997 *Internal Risk Assessment Forum Review Draft of the Guidance on Health Risk Assessment of Chemical Mixtures*. The Technical Panel revised the document in accordance with comments received during the July 1997 review. In June 1998 the Forum sponsored an Agency review and colloquium. Over the next months the Technical Panel worked with commenters to address issues raised during the 1998 colloquium to prepare the document for external peer review. It was determined at this time that the broad principles and concepts put forth in the 1986 Guidelines remained applicable, but needed more detail. As a result it was determined that the document would supplement, and not replace the 1986 Guidelines. An external peer review was convened in May 1999. Twelve independent experts representing consulting, academia, industry, the U.S. Department of Health Agency for Toxic Substances and Disease Registry, and the TNO Nutritional and Food Research Institute of the Netherlands, reviewed the revised supplementary document dated April 1999. The experts provide comments that reflected their experience and expertise in toxicology, mechanistic and pharmacokinetic modeling, statistics, and risk assessment (risk assessment of chemical classes, of complex and unidentifiable mixtures, and of multi-chemical exposures at Superfund sites). Their comments are documented in the report entitled, *Report of the Peer Review Workshop on the Guidance for Conducting Health Risk*

Assessments of Chemical Mixtures (Eastern Research Group Inc., 1999). During the summer of 1999 the Technical Panel considered comments from the external experts and from the Forum in revising and reorganizing the supplementary document. This series of internal and external reviews has ensured that the supplementary guidance is consistent with related science and Agency guidance developments.

After an abbreviated overview of the background and scope, the *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* document puts forth the risk assessment paradigm for mixtures. This paradigm begins with problem formulation, then briefly discusses hazard identification, dose-response assessment, exposure, and risk characterization. The document is organized according to the type of data available to the risk assessor, ranging from data-rich to data-poor situations. (See Figure 2-1). Procedures are described for assessment using data on the mixture of concern, data on a toxicologically similar mixture, and data on the mixture component chemicals. The state of the science varies dramatically for these three approaches. The whole-mixture procedures are most advanced for assessing carcinogenic risk, mainly because of the long use of in vitro mutagenicity tests to indicate carcinogenic potency. In vitro test procedures for noncancer endpoints are still in the pioneering stage. In contrast, the component-based procedures, particularly those that incorporate information on toxicologic interactions, are most advanced for noncarcinogenic toxicity. No single approach is recommended in this supplementary guidance. Instead, guidance is given for the use of several approaches depending on the nature and quality of the data. The appendices contain definitions, a discussion on toxicologic interactions and pharmacokinetic models, and a reprint of the 1986 Guidelines.

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EXECUTIVE SUMMARY

This supplementary guidance document is organized according to the type of data available to the risk assessor, ranging from data rich to data poor situations. This organization reflects the approaches to chemical mixture risk assessment recommended in the 1986 *Guidelines for the Health Risk Assessment of Chemical Mixtures* (Appendix A). This document describes more detailed procedures for chemical mixture assessment using data on the mixture of concern, data on a toxicologically similar mixture, and data on the mixture component chemicals. The state-of-the-science varies dramatically for these three approaches. It is recommended that the risk assessor implement several of the approaches that are practical to apply and evaluate the range of health risk estimates that are produced.

This document suggests that the selection of a chemical mixture risk assessment method follows the outline in the flow chart shown in Figure 2-1, which begins with an assessment of data quality and then leads the risk assessor to selection of a method through evaluation of the available data. The major concerns for the user are whether the available data are on components or whole mixtures, whether the data are composed of either similar components or similar mixtures that can be thought of as acting by similar toxicologic processes, and whether the data may be grouped by emissions source, chemical structure, or biologic activity. Method-specific user fact sheets for quantitative risk assessment can be found in Sections 2.5 and 2.6 and are intended to provide a concise overview of each currently available method. These fact sheets provide the following information relative to the risk assessment approach:

- Type of Assessment
- Data Requirements
- Section(s)
- References
- Strategy of Method
- Ease of Use
- Assumptions
- Limitations
- Uncertainties

In Figure 2-1, an evaluation of the data may lead the user to decide that only a qualitative analysis should be performed. This generally occurs in cases where data quality is poor, inadequate quantitative data are available, data on a similar mixture cannot be classified as

"sufficiently similar" to the mixture of concern, exposures cannot be characterized with confidence, or method-specific assumptions about the toxicologic action of the mixture or of its components cannot be met. When this occurs, the risk assessor can still perform a qualitative assessment that characterizes the potential human health impacts from exposure to that mixture. Such a risk characterization should discuss each element of the risk assessment paradigm, including available information on the mixture itself, on its components, and on potential interactions among the components. Any information on fate and transport of the mixture that would affect its final composition at the time of exposure should be noted.

The assessment of chemical mixtures is an area of active scientific investigation. As new information relevant to health risk from exposure to chemical mixtures becomes available, additional guidance documents will be published.

1. INTRODUCTION

1.1. BACKGROUND

Although some potential environmental hazards involve significant exposure to only a single compound, most instances of environmental contamination involve concurrent or sequential exposures to a mixture of compounds that may induce similar or dissimilar effects over exposure periods ranging from short-term to lifetime. For the purposes of this guidance document, a mixture will be defined as any combination of two or more chemical substances, regardless of source or of spatial or temporal proximity, that can influence the risk of chemical toxicity in the target population (U.S. EPA, 1986). In some instances, the mixtures are highly complex, consisting of scores of compounds that are generated simultaneously as by-products from a single source or process (e.g., coke oven emissions and diesel exhaust). In other cases, complex mixtures of related compounds are produced as commercial products (e.g., PCBs, gasoline and pesticide formulations) and eventually released into the environment. Another category of mixtures consists of compounds, often unrelated chemically or commercially, that are placed in the same area for disposal or storage, and have the potential for combined exposure to humans. Multichemical exposures are ubiquitous, including air and soil pollution from municipal incinerators, leakage from hazardous waste facilities and uncontrolled waste sites, and drinking water containing chemical substances formed during disinfection.

To address concerns over health risks from multichemical exposures, the U.S. Environmental Protection Agency, hereafter referred to as EPA, issued *Guidelines for the Health Risk Assessment of Chemical Mixtures* in 1986 (U.S. EPA, 1986) (Appendix A). Those Guidelines described broad concepts related to mixture exposure and toxicity and included few specific procedures. In 1989, EPA published guidance for the Superfund program on hazardous waste that gave practical steps for conducting a mixtures risk assessment (U.S. EPA, 1989a). Also in 1989, EPA published the revised document on the use of Toxicity Equivalence Factors for characterizing health risks of the class of chemicals including the dibenzo-dioxins and dibenzofurans (U.S. EPA, 1989b). In 1990, EPA published a Technical Support Document to provide more detailed information on toxicity of whole mixtures and on toxicologic interactions (e.g., synergism) between chemicals in a binary (two-chemical) mixture (U.S. EPA, 1990). The concept of toxicologic similarity was also discussed.

As more waste sites were evaluated for mixtures risks, it became apparent that the exposure scenarios for these sites were extremely diverse. Moreover, the quality and quantity of pertinent information available for risk assessment varied considerably for different mixtures. Such difficulties continue. Occasionally, the chemical composition of a mixture is well characterized, levels of exposure to the population are known, and detailed toxicologic data on

the mixture are available. Most frequently, some components of the mixture are unknown, exposure data are uncertain or vary over time, and toxicologic data on the known components of the mixture are limited. Consequently, this document has been developed to supplement the earlier guidance documents and is organized according to the type of data available to the risk assessor, ranging from data-rich to data-poor situations. Procedures are described for assessment using data on the mixture of concern, data on a toxicologically similar mixture, and data on the mixture component chemicals. The state of science varies dramatically for these three approaches. The whole-mixture procedures are most advanced for assessing carcinogenic risk, mainly because of the long use of in vitro mutagenicity tests to indicate carcinogenic potency. In vitro test procedures for noncancer endpoints are still in the pioneering stage. In contrast, the component-based procedures, particularly those that incorporate information on toxicologic interactions, are most advanced for noncarcinogenic toxicity.

Mixture risk assessments usually involve substantial uncertainties. If the mixture is treated as a single complex substance, these uncertainties range from inexact descriptions of exposure to inadequate toxicity information. When viewed as a simple collection of a few component chemicals, the uncertainties include the generally poor understanding of the magnitude and nature of toxicologic interactions, especially those interactions involving three or more chemicals. Because of these uncertainties, the assessment of health risk from chemical mixtures should include a thorough discussion of all assumptions and the identification, when possible, of the major sources of uncertainty. No single approach is recommended in this supplementary guidance. Instead, guidance is given for the use of several approaches depending on the nature and quality of the data.

1.2. OVERVIEW

The primary purpose of this document is to generate a consistent Agency approach for assessing health risks from exposures to multiple chemicals, denoted in this guidance by the general term “mixtures.” The resulting mixtures risk assessments are intended to assist decision makers by characterizing health risks for the particular exposure conditions of interest. Because exposure scenarios and the available supporting data are highly diverse, this document has been developed as a procedural guide that emphasizes broad underlying principles of the various science disciplines (environmental chemistry, toxicology, pharmacology, statistics) necessary for providing information on the relationship between multichemical exposure and potential health effects. Specific approaches to be used for the evaluation of the various kinds of mixture data are also discussed.

This document addresses only risks to human health from multichemical exposures. Ecological effects are beyond its scope, even though many of the procedures might be adaptable

to ecological risk assessment from multiple stressors. Because other Agency guidelines exist that address exposure assessment and specific toxic endpoint evaluations, this guidance focuses on procedures for dose-response assessment and risk characterization.

It is not the intent of this guidance document to regulate any social or economic aspects concerning risk of injury to human health or the environment caused by exposure to a chemical agent(s). All such action is addressed in specific statutes and federal legislation and is independent of this guidance.

This guidance document represents a supplement to the original Guidelines of 1986 and is intended to reflect the evolutionary scientific development in the area of chemical mixtures risk assessment. New guidance has been provided that gives more specific details on the nature of the desired information and the procedures to use in analyzing the data. Among these are methods for using whole-mixture data on a toxicologically similar mixture, methods for incorporating information on toxicologic interactions to modify a Hazard Index (HI), and generalized procedures for mixtures involving classes of similar chemicals. There are also expanded discussions of the concerns when using only whole-mixture data as well as when using only data on the individual chemical components.

The assessment of chemical mixtures is an area of active scientific investigation. Some of the procedures herein for chemical mixtures have had little or no application to date in actual health risk assessments. Their use is encouraged, along with research on new procedures to improve or replace those discussed here. As new information relevant to health risk from exposure to chemical mixtures becomes available, additional guidance documents will be published.

2. APPROACH TO RISK ASSESSMENT OF CHEMICAL MIXTURES

2.1. THE RISK ASSESSMENT PARADIGM FOR MIXTURES

Human health risk assessments done by EPA generally follow the paradigm established by the National Academy of Sciences (NRC, 1983). This paradigm describes a group of interconnected processes for performing a risk assessment that include hazard identification, dose-response assessment, exposure assessment, and risk characterization. These four parts of the paradigm are used as the foundation for the procedures presented in this guidance. Preamble to all is problem formulation, which is defined in EPA's (1998a) Ecological Risk Assessment Guidelines as "a process for generating and evaluating preliminary hypotheses about why...effects have occurred or may occur." This EPA guidance for assessing risks from exposures to chemical mixtures begins with problem formulation as the initial step; much of the information about this key step has been adapted from the Ecological Risk Assessment Guidelines, and the reader is referred to Chapter 3 of that document for a more comprehensive discussion (U.S. EPA, 1998a).

2.1.1. Problem Formulation

Problem formulation, which provides the foundation for the entire risk assessment, consists of three initial steps: (1) evaluate the nature of the problem, (2) define the objectives of the risk assessment, and (3) develop a data analysis and risk characterization plan. The quality, quantity, and pertinence of information will determine the course of problem formulation. It concludes with three products: (1) selection of assessment endpoints, (2) review of the conceptual models that describe the relationship between exposure to a mixture of chemicals and risk, and (3) adjusting the analytic plan. (The pertinence of the information that is available at the outset of the assessment, in combination with the assessment objectives, will identify the types of information that should be collected through the analytic plan.) Ideally, the problem is formulated jointly by risk analysts and risk managers. While the steps and outcomes associated with problem formulation are presented separately, experiences from ecological applications and Superfund site assessments show the process to be frequently interactive and iterative rather than linear.

2.1.2. Hazard Identification and Dose-Response Assessment

In *hazard identification*, available data on biological endpoints are used to determine if a material is likely to pose a hazard to human health. These data are also used to define the type of potential hazard (e.g., does the material induce tumor formation or act as a kidney toxicant). In the *dose-response assessment*, data (most often from animal studies and occasionally from

human studies) are used to estimate the amount of material that may produce a given effect in humans. The risk assessor may calculate a quantitative dose-response relationship usable for low-dose exposure, often by applying mathematical models to the data.

2.1.3. Exposure

The *exposure assessment* seeks to determine the extent to which a population is exposed to the material. Exposure assessment uses available data relevant to population exposure, such as emissions data, measurement of the material in environmental media, and biomarker information. Fate and transport of the material in the environment, as well as media, pathways, and routes of exposure, may all be considered in the exposure assessment. Data limitations on the environmental concentrations of interest often necessitate the use of modeling to provide relevant estimates of exposure.

2.1.4. Risk Characterization and Uncertainty

Risk characterization is the integrating step in the risk assessment process that summarizes assessments of effects on human health and ecosystems and assessments of exposure from multiple environmental media, identifies human subpopulations or ecological species at elevated risk, combines these assessments into characterizations of human and ecological risk, and describes the uncertainty and variability in these characterizations. In March 1995, the Administrator of EPA issued the *Policy for Risk Characterization at the U.S. Environmental Protection Agency* (U.S. EPA, 1995). The purpose of this policy statement was to ensure that critical information from each stage of a risk assessment be presented in a manner that provides for greater clarity, transparency, reasonableness, and consistency in risk assessments. Most of the 1995 *Policy for Risk Characterization at the U.S. EPA* was directed toward assessment of human health consequences of exposures to an agent. Key aspects of risk characterization identified in the 1995 *Policy for Risk Characterization at the U.S. EPA* include these: bridging risk assessment and risk management, discussing confidence and uncertainties, and presenting several types of risk information. Another publication, *Science and Judgment in Risk Assessment* (NRC, 1994), produced primarily for implementation of the 1990 Amendment to the Clean Air Act but applicable more generally, emphasized that the goal of risk characterization is to provide understanding of the type and magnitude of potential adverse effects of an agent under the particular circumstances of its release.

2.1.5. Incorporating the Paradigm Into Mixtures Guidance

EPA regularly publishes guidelines to provide for consistency of application and communication of risk assessment. Guidelines were published in 1986 on assessment of the

following areas: exposure, developmental effects, germ cell mutagenicity, carcinogenic effects, and chemical mixtures (U.S. EPA, 1986, 1987). Since that time, the Agency has revised some of these Guidelines and also published new Guidelines. These include Guidelines on developmental toxicity (U.S. EPA, 1991a), exposure assessment (U.S. EPA, 1992), cancer (proposed revisions) (U.S. EPA, 1996a), reproductive toxicity (U.S. EPA, 1996c), and neurotoxicity (U.S. EPA, 1998b). All of the EPA guidelines for human health risk assessment incorporate the four parts of the NAS paradigm.

For this supplemental guidance on the risk assessment of chemical mixtures, the four parts of the paradigm are interrelated and will be found within the assessment techniques that are presented. For some methods described herein, assessment of dose-response relies both on decisions in the area of hazard identification and on assessment of potential human exposures. For mixtures, the use of pharmacokinetics data and models in particular differs from single-chemical assessment, where they are often part of the exposure assessment. For mixtures, the dominant mode of toxicologic interaction is the alteration of pharmacokinetic processes, which strongly depends on the exposure levels of the mixture chemicals. In this guidance, there has been no effort to categorize methods strictly or arbitrarily into one part of the paradigm. The methods are organized instead according to the type of available data. In general, risk characterization takes into account both human health and ecological effects, and also assesses multiroute exposures from multiple environmental media. This guidance focuses only on the human health risk assessment for chemical mixtures and only discusses multiroute exposures in terms of conversions from dermal to oral.

2.2. PROCEDURE FOR SELECTING A RISK ASSESSMENT METHOD

2.2.1. Introduction

The 1986 *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986) (Appendix A) recommend three approaches to quantitative health risk assessment of a chemical mixture, depending upon the type of available data. In the first approach, toxicity data on the mixture of concern are available; the quantitative risk assessment is done directly from these preferred data. In the second approach, when toxicity data are not available for the mixture of concern, the Guidelines recommend using toxicity data on a “sufficiently similar” mixture. If the mixture of concern and the proposed surrogate mixture are judged to be similar, then the quantitative risk assessment for the mixture of concern may be derived from health effects data on the similar mixture. Finally, the third approach is to evaluate the mixture through an analysis of its components, e.g., using dose addition for similarly acting chemicals and response addition for independently acting chemicals. These procedures include a general assumption that interaction effects at low dose levels either do not occur at all or are small enough to be

insignificant to the risk estimate. The Guidelines recommend the incorporation of interactions data when available, if not as part of the quantitative process, then as a qualitative evaluation of the risk.

No single approach is recommended in this guidance document. Instead, guidance is given for the use of several approaches depending on the nature and quality of the available data, the type of mixture, the type of assessment being made, the known toxic effects of the mixture or of its components, the toxicologic or structural similarity of mixtures or of mixture components, and the nature of the environmental exposure. The approaches presented herein represent a mix of well-known, routine methods with several newer, less well-established techniques. As a collection, they provide the risk assessor with a number of reasonable options for evaluating risk for chemical mixtures.

2.2.2. Steps for Selection

This guidance suggests that the selection of a chemical mixture risk assessment method follow the outline in the flow chart shown in Figure 2-1, which begins with an assessment of data quality and then leads the risk assessor to selection of a method through evaluation of the available data. The major concerns for the user are whether the available data are on components or whole mixtures, whether the data are composed of either similar components or similar mixtures that can be thought of as acting by similar toxicologic processes, whether the mixture components act by the same mode of action or are functionally independent, or whether the data may be grouped by emissions source, chemical structure, or biologic activity.

This document is organized around the decision points in Figure 2-1, so that the user can refer to specific sections and find guidance on the issues to consider when working through the flow chart. Appendix B also offers the user a number of definitions to help clarify the terminology that is unique to chemical mixtures risk assessment. Table B-1 presents chemical mixture definitions in terms of specific criteria including the complexity of the mixture, similarity of biologic activity, similarity of chemical structure or mixture composition, the environmental source of the mixture, toxic endpoint, etc. Table B-2 provides definitions for terms that are used to describe various types of toxicologic interactions including forms of additivity, antagonism, synergism, and other toxicologic phenomena.

Method-specific user fact-sheets in Sections 2.5 and 2.6 are intended to provide a concise overview of each currently available method. These fact-sheets provide the following information relative to the risk assessment approach:

- Type of Assessment: distinguishes whether the approach is a dose-response assessment or whether it combines dose response and exposure information to perform a risk characterization.

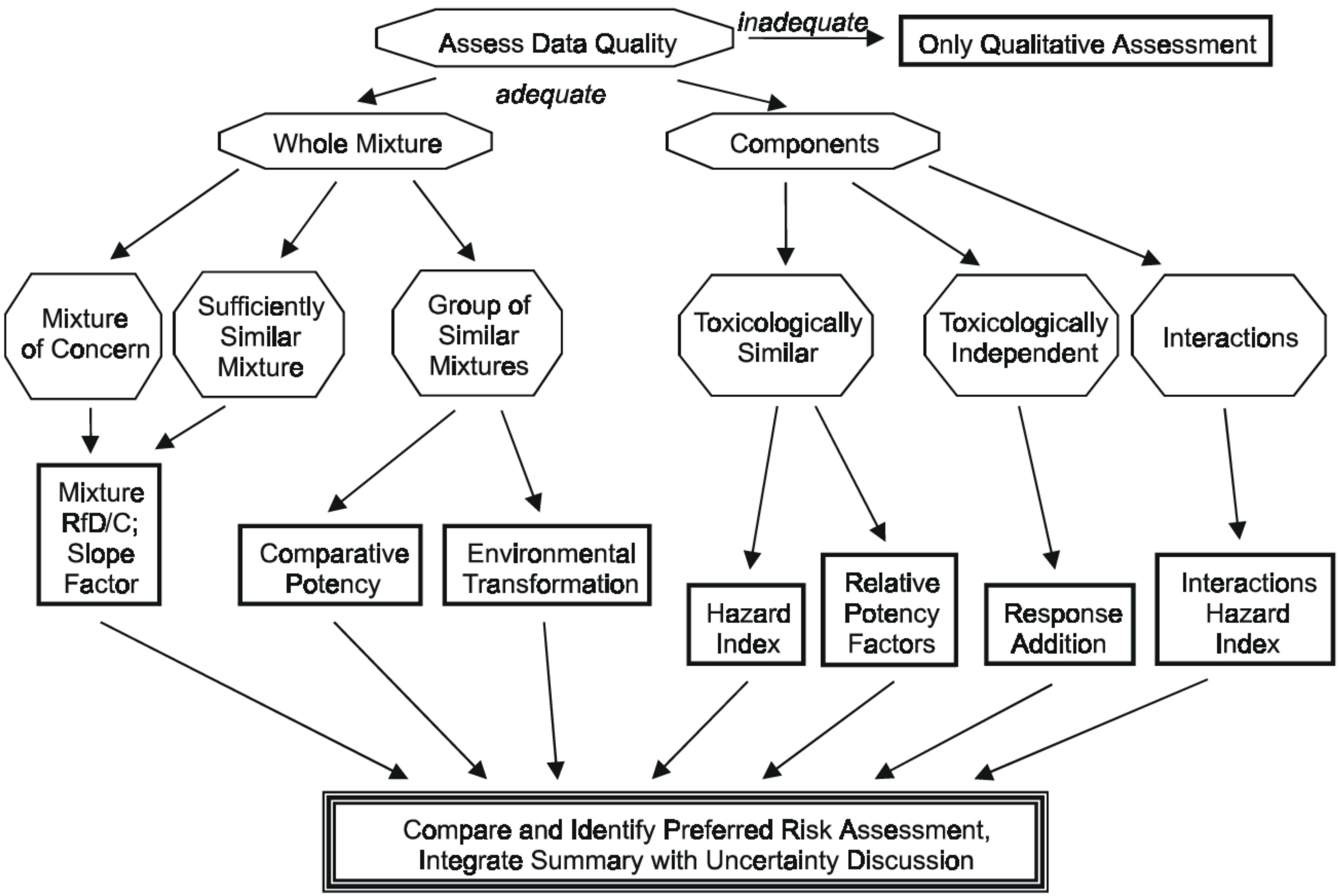

Figure 2-1. The different types of mixtures assessments based on the availability and quality of the data. All possible assessment paths should be performed.

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- Data Requirements: details the types and amount of data that are needed to carry out the procedure.
- Section(s): refers the user to sections of this document that provide greater detail on the approach.
- References: cites reports or publications in which the approach has been applied in practice or indicates that this is a new procedure.
- Strategy of Method: provides concise directions on how the calculations are performed.
- Ease of Use: gives a sense of how much effort, expertise, and data are required in order to apply the approach.
- Assumptions: lists the toxicologic or statistical assumptions that are inherently made when the data are treated by applying the approach; the user can then decide if the approach is appropriate for the available data.
- Limitations: suggests problems the user may encounter relative to data gaps or quality deficiencies, and statistical modeling requirements or goodness-of-fit issues.
- Uncertainties: indicates unknown elements of the analysis that should be considered and characterized in the presentation of the risk assessment (e.g., data are not available, mode of action is unknown, scientific judgments are made, exposures are not well characterized, extrapolations are made, etc.).

Following an assessment of data quality, the first major distinction addressed in Figure 2-1 is whether the type of available data is whole mixture data or mixture component information. This distinction points the risk assessor toward methods that are available for these specific types of data. Methods available for whole mixtures then depend on whether there is information directly available on the mixture of concern or only on sufficiently similar mixtures or groups of similar mixtures. Methods available for component data then depend on whether there are interactions data available or whether the components act with a similar mode of action or are toxicologically independent. In these cases, the outcome is a quantitative assessment with a complete risk characterization and uncertainty discussion presented.

Figure 2-1 is deceptively simple, however, as many of the issues that are represented in the diagram require the use of scientific judgment or data that may not be readily available. In addition, there will often be mixtures for which there exist both whole-mixture and component data, so that the choice of method will not be clear (for example, both epidemiologic data and component toxicity data exist for evaluation of health effects from exposure to chlorinated drinking water). Furthermore, the true toxicologic mechanism of action (see Section 2.2.3) is rarely known for a given mixture or even for most of its components; thus the judgments that are made of toxicologic similar action or independence of action, for example, will be uncertain. It is recommended, therefore, that the risk assessor implement several of the approaches that are practical and evaluate the range of health risk estimates that are produced.

2.2.3. Key Concepts

There are several concepts that must be understood in order to evaluate a chemical mixture (see Appendix B). The first is the role of toxicologic similarity which, in this document, is considered along a continuum of information. The term mode of action is defined as a series of key events and processes starting with interaction of an agent with a cell, and proceeding through operational and anatomical changes causing disease formation. "Mode" of action is contrasted with "mechanism" of action, which implies a more detailed understanding and description of events, often at the molecular level, than is meant by mode of action. The specific term *toxicologic similarity* represents a general knowledge about the action of a chemical or a mixture and can be expressed in broad terms such as at the target organ level in the body (e.g., enzyme changes in the liver). In this document, assumptions about toxicologic similarity are made in order to choose among risk assessment methods. In general, we assume a similar *mode of action* across mixtures or mixture components and, in some cases, this requirement may be relaxed to require that these chemicals act only on the *same target organ*.

The second key concept in understanding mixtures risk assessment is the assumption of similarity or, in contrast, independence of action. The term *sufficiently similar mixture* refers to a mixture that is very close in composition to the mixture of concern, such that differences in their components and their proportions are small; the risk assessor can then use the data from the sufficiently similar mixture to make a risk estimate about the mixture of concern. The term *similar components* refers to the single chemicals within a mixture that act by the same mode of action and may have comparable dose-response curves; the risk assessor can then apply a component-based approach that uses these characteristics to form the basis of the risk assessment. The term *group of similar mixtures* refers to chemically related classes of mixtures that act by a similar mode of action, have closely related chemical structures, and occur together routinely in environmental samples, usually because they are generated by the same commercial process; the risk assessor can use what is known about the shifts in chemical structure and relative potency of the components to perform a risk assessment. Finally, the term *independence of action* is defined as mixture components that cause different kinds of toxicity, or effects in different target organs; the risk assessor may then combine the probabilities of toxic effects for the individual components.

Another key concept for this document is the understanding of language referring to toxicologic interactions, which is defined here as any toxic responses that are greater than or less than what is observed under an assumption of *additivity*. The term *additivity* is used when the effect of the combination of chemicals can be estimated directly from the sum of the scaled exposure levels (dose addition) or of the responses (response addition) of the individual components. There are a myriad of terms (see Appendix B, Table B-2) that represent various

kinds of interaction effects (e.g., inhibition, antagonism, masking). The most common and general of these refer to effects that are greater than additive (i.e., synergistic) or less than additive (i.e., antagonistic).

2.2.4. Qualitative Assessments

In Figure 2-1, an evaluation of the data may lead the user to decide that only a qualitative analysis should be performed. This generally occurs in cases where data quality is poor, there are inadequate quantitative data available, data on a similar mixture cannot be classified as "sufficiently similar" to the mixture of concern, exposures cannot be characterized with confidence, or method-specific assumptions about the toxicologic action of the mixture or of its components cannot be met. When this occurs, the risk assessor can still do a qualitative assessment that characterizes the potential human health impacts from exposure to that mixture. Such a risk characterization should discuss each element of the risk assessment paradigm, including available information on the mixture itself, on its components, and on potential interactions among the components. Any information on fate and transport of the mixture that would affect its final composition at the time of exposure should be noted.

2.2.5. Defaults

The development of a risk assessment for a chemical mixture will generally involve the examination of complex exposures and toxicities and the application of specific methods as well as scientific judgment. This process necessarily involves a thorough examination and discussion of the uncertainties, limitations, and assumptions inherent in exposure assessment, fate and transport, uptake and pharmacokinetics, and the magnitude and nature of toxicity and toxicant interactions. Because of the complexity of considerations that must be undertaken to develop a chemical mixtures health risk assessment, it is not practical to recommend a clear listing of default procedures that covers all cases. In many cases, information gaps will be too substantial to allow use of defaults, so that only a qualitative risk assessment can be performed. Nonetheless, for some restricted situations, default values and methods can be recommended. This section outlines the philosophy underlying their choice.

For low exposure levels when no interactions information is available, default methods using an additivity assumption are given. For the component chemicals in a mixture that show dissimilar toxicity, response addition (Sections 2.6.2, 4.1, and 4.5) is recommended. For the component chemicals that show similar toxicity, dose addition (Sections 2.6.1, 4.1, 4.2, and 4.4) is recommended. Under dose addition, the general procedure is to scale the doses of the components by their relative potency and add the scaled doses together; the mixture response is then estimated for the combined dose. Under response addition, the general procedure is to first

determine the risks per the exposure for the individual components; the mixture risk is then estimated by adding the individual risks together. These processes are fundamentally different and require different assumptions of the data in order for them to be used appropriately. Finally, if interactions data are available, the default recommendation is that they be incorporated into the risk assessment by using the interaction-based Hazard Index (HI) (Sections 2.6.3, 4.1, and 4.3).

Dose addition is the default approach in situations where the dose for each individual component is at a level at which effects are not expected to occur, be observable, or be of concern; however, when the doses are combined, effects of concern may be expected or observed in response to the higher dose level of the mixture. A method based on dose addition that has been used most often by EPA is the HI, where HI < 1 indicates a mixture exposure of no significant concern (U.S. EPA, 1989a). True dose addition is applied by scaling the potencies of all the components in the mixture with the same mechanism of action to an index chemical, adding the scaled doses together to give the equivalent dose in terms of the index chemical, and using the index chemical's dose-response curve to estimate the response for the equivalent total mixture dose. Dose addition is different from response addition because two assumptions are made: that all of the components have similar uptake, pharmacokinetics, and toxicologic processes; and that the dose-response curves of the components have congruent or similar shape (Teuschler and Hertzberg, 1995). This means that, for equal effects, the dose of one component is a constant multiple of the dose of a second component.

The interaction-based HI is the default approach for using interactions data to modify simple dose addition. This approach uses binary interactions data for the components of the mixture to modify the HI. The factors that are used include the interaction magnitude at low doses, the toxicity of each component relative to each other component, the weight of evidence of the interactions data, and the relative proportions of the components in the mixture.

Response addition is the default approach when the component chemicals are functionally independent. It is most often applied when an effect that is of concern is expected to be present at low dose levels for each of the component chemicals, even though it is highly unlikely to be observable at these low levels in either epidemiologic or toxicologic studies; the mixture risk is then usually approximated by the sum of the individually low risks of the independently acting component chemicals. For example, response addition has often been used for the risk assessment of mixtures of carcinogens (Gaylor et al., 1997; U.S. EPA, 1989a). Response addition is different from dose addition in that it does not assume similar kinetics or a similar mode of action and does not assume that the dose-response curves have similar shape. It assumes that the components of the mixture are functionally independent of one another at low exposure levels (Mumtaz and Hertzberg, 1993), so that the risks may be added together (see Section 4.5 for details on interpretation and calculation). Because response addition does not

require a similar mode of action across the chemicals in the mixture, it allows for combining risks across chemicals even if they have different types of endpoints. An example is the combined risk of any kind of reproductive toxicity for a set of chemicals with different modes of action.

2.3. DATA QUALITY ASSESSMENT

The first consideration in Figure 2-1 is the assessment of data quality relative to its relevancy, completeness, quantitative nature, and certainty in three areas: exposure information, health effects information, and information on interactions. Table 2-1 presents a classification scheme for assessing the quality and nature of the available mixtures data. Consideration of the factors presented in Table 2-1 can be used to guide the risk assessor through Figure 2-1. This evaluation can assist the decision of whether to quantify the risk (the first step in Figure 2-1), and can be included in a discussion of overall quality of the risk assessment. Usually a classification of “FAIR” or better is required for quantitative risk assessment. For example, a “GOOD” classification for each of exposure information, health effects information and information on interactions would lead the risk assessor to consider the data quality to be adequate for quantification, with good data available for both the exposure and toxicity aspects of the mixture of concern. Figure 2-1 would then guide the risk assessor to perform a risk assessment directly on the mixture of concern by calculating, for example, a toxicity value for the mixture, such as a Reference Dose (RfD) or slope factor. A “POOR” classification for one or more of these categories would likely lead the risk assessor to decide that data quality was inadequate; in this case, Figure 2-1 directs the risk assessor to perform only a qualitative risk assessment. With “FAIR” information on each of exposure, health effects, and interactions, the risk assessor would conclude that data quality was adequate to estimate both the exposure and toxicity of the components of the mixture, and furthermore to use the available interactions data on the components in the assessment. Under these conditions, Figure 2-1 indicates that an interaction-based HI approach would be appropriate. It is the purview of the risk assessor to decide at what point the validity of the risk assessment is compromised by the data quality to such a degree that only a qualitative assessment should be performed.

2.3.1. Quality of Exposure Information

Exposure information ideally includes all data needed to characterize the human exposure to the mixture of concern from the point of environmental release to the point of human intake. There are several details needed to quantify exposure to chemical mixtures; these include:

Table 2-1. Classification scheme for the quality of available mixtures data[a]

		Exposure Information[b]
GOOD	–	Monitoring information either alone or in combination with modeling information is sufficient to accurately characterize human exposure to the mixture or its components.
	–	Modeling information is sufficient to reasonably characterize human exposure to the mixture or its components.
FAIR	–	Exposure estimates for some components are lacking, uncertain, or variable. Information on health effects or environmental chemistry suggests that this limitation is not likely to substantially affect the risk assessment.
	–	Not all components in the mixture have been identified, or levels of exposure are highly uncertain or variable. Information on health effects or environmental chemistry is not sufficient to assess the effect of this limitation on the risk assessment.
POOR	–	The available exposure information is insufficient for conducting a risk assessment.
		Health Effects Information
GOOD	–	Full health effects data are available and relatively minor extrapolation is required.
	–	Full health effects data are available but extensive extrapolation is required for route or duration of exposure or for species differences. These extrapolations are supported by pharmacokinetic considerations, empirical observations, or other relevant information.
FAIR	–	Full health effects data are available, but extensive extrapolation is required for route or duration of exposure or for species differences. These extrapolations are not directly supported by the information available.
	–	Certain important health effects data are lacking and extensive extrapolations are required for route or duration of exposure or for species differences.
POOR	–	A lack of health effects information on the mixture and its components in the mixture precludes a quantitative risk assessment.
		Information on Interactions
GOOD	–	Assessment is based on toxicologic data on the mixture of concern.
	–	Assessment is based on data on a sufficiently similar mixture.
FAIR	–	Quantitative interactions of all components are well characterized.
	–	The assumption of additivity is justified based on the nature of the health effects and on the number of component compounds.
POOR	–	Interactions information is inadequate, an assumption of additivity cannot be justified, and no quantitative risk assessment can be conducted.

[a]See text for discussion of sufficient similarity, adequacy of data, and justification for additivity assumptions.
[b]See the Agency's guidelines for exposure assessment (U.S. EPA, 1992) for more complete information on performing exposure assessments and evaluating the quality of exposure data.

- Concentration of the chemical mixture in the medium/media of concern at the point(s) of human contact

- The duration and frequency of exposure should be developed from repeated measurements or validated models of environmental fate in media to which individuals are exposed, as well as human activity pattern data. The media concentrations should be determined at the points of human exposure. If the exposure data are limited, the analyst should address the degree to which the data

represent the environmental chemical mixture over space and time. Environmental transformation of the mixture over time is a key concern.

- Analytic chemistry

  The analyst should consider both the accuracy and reliability of the measurement techniques and determine if all of the components have been identified (i.e., are there unidentified components of the mixture?). The analyst should also determine if the key environmental reactions have been identified and reaction rates measured (e.g., environmental half-life) that govern the fate of the mixture. If components of the environmental mixture have not been detected analytically, the analyst should describe if and how they were included in the assessment (e.g., the compounds were assumed to be present at one-half the detection limit).

- Uptake from the environment

  The analyst should examine the bioavailability of the mixture for the medium and route of concern. The ideal data set would be derived from well-conducted studies that measure either the entire mixture or all the components in the pertinent exposure media and over the timeframe of concern. (The ideal data may be derived from accurate analytic measurements at points of human contact or from validated environmental fate models.) The magnitude of the human exposure would be measured or modeled on the basis of human activity patterns. Finally, the bioavailability of the mixture or the components would be known. Unfortunately, a complete data set is rarely available. The analyst should identify (and perhaps quantify) uncertainty based on imperfect analytic methods (e.g., some constituents may not be characterized by the analytic technique that represents the current state of the science), extrapolations between concentrations at measurement points and points of human exposure, incompletely understood transformation reactions to the mixture in the environment, and bioavailability. Each of these uncertainties in the risk assessment should be discussed and accounted for in the final risk characterization.

2.3.2. Quality of Health Effects Information

Health effects information includes both hazard identification and dose-response data on the complex mixture, a similar mixture, or the components of the mixture. The best data would be human epidemiologic or human clinical data directly on the complex mixture for which the health effects of concern are causally linked to the mixture exposure and a dose-response relationship can be established for the exposure route of interest. Unfortunately, such high-quality direct information is rarely available, so the risk assessor usually performs one or more extrapolations. Examples of such extrapolations include using animal data to project potential human health effects, using inhalation data to predict risks from oral exposure, using component data to estimate risks for the complex mixture, and using data from short-term human clinical

studies or subchronic animal bioassays to project human health risks from chronic exposure. Each of these extrapolations introduces uncertainty into the risk assessment that should be discussed and accounted for in the final risk characterization.

2.3.3. Quality of Interactions Information

Interactions information includes any data indicating that the toxicologic action of the complex mixture is greater than or less than what might be expected from exposure to a colleciton of individual components of the mixture. Thus, human or animal data directly on the whole mixture implicitly provides interactions information for use in risk assessment. However, since such data are rarely available, the risk assessor must often rely on component information, the vast majority of which is laboratory toxicity data on binary combinations of chemicals (Teuschler and Hertzberg, 1995). The quality of interactions data, whether it be data on the complex mixture, a sufficiently similar mixture, or simple combinations of the components, can be judged according to the strength of evidence for three criteria. First, there should be adequate toxicity data that not only provide information on dose response, but also on the mechanism of action for the mixture. Second, interactions data should be for the same route of exposure as the mixture of concern. Furthermore, when data on several different component mixtures are evaluated, these data should be from comparable studies, such as the same species, same endpoint of concern, similar laboratory conditions, or comparable study duration. Finally, observed interactions data that are usable for risk assessment purposes should be toxicologically significant (i.e., show definite adverse effects). The strength of the evidence for each of these criteria should be discussed and accounted for in the final risk characterization.

2.4. CHEMICAL MIXTURE EXPOSURE ASSESSMENT ISSUES

While this guidance document is intended to serve risk assessors primarily by informing them of dose-response and risk characterization methods associated with exposures to chemical mixtures, the purpose of this section is to highlight additional exposure issues of a *general* nature that should be considered when developing a risk assessment for chemical mixtures. The issues presented in this section should be considered in addition to those normally followed in an exposure assessment. The Agency's primary guidance in this area is the Exposure Assessment Guidelines (U.S. EPA, 1992); however, that document primarily focuses on issues pertaining to single-chemical exposures. Other, more specific exposure assessment issues involving multiple chemicals will be discussed by the Agency more comprehensively in separate future efforts (e.g., the EPA's Risk Assessment Forum is developing a cumulative risk assessment framework as this guidance goes to press). While there are other important issues related to exposures to chemical

mixtures, three critical areas will be discussed briefly here: environmental fate, temporal patterns of exposure, and routes of exposure.

The wide diversity in mixture compositions and site characteristics precludes any recommendation for a single approach for site-specific modification of the mixture assessment. Through examples, some steps that should be considered can be articulated. The example in Section 3.4 demonstrates some of the considerations that should be part of such a modification. Other modifications based on the exposure and mixture characteristics are encouraged, as long as they are clearly described and supported with plausible concepts and empirical measurements. Clearly, the analyst should report the significance of any assumptions utilized as well as the potential uncertainty and variability associated with the exposure modifications developed for the risk assessment.

2.4.1. Environmental Fate and Transport

The composition and quantity of a mixture of chemicals may change after release into the environment. The environmental fate of chemical mixtures released into the environment can be conceptualized as being composed of three *interrelated* components: (1) transport through an individual compartment (e.g., atmospheric dispersion); (2) transfer between environmental compartments (i.e., partitioning); and (3) transformation mediated by biological, chemical, or physical processes (e.g., weathering) (Crawford-Brown, 1997, Chapter 2). Even though the environmental processes that occur within these three components of environmental fate are not unique to chemical mixtures, the analyst should assess compositional and quantitative changes that may occur to the chemical mixture of interest in the environment (particularly with respect to the time from release to exposure), and the impact these will have on exposure and toxicity.

This is particularly important when considering the appropriateness or relevance of an analytic measurement of quantity or composition of a chemical mixture; the analyst needs to consider the possible changes to the mixture between the time the measurement was conducted and the time over which exposures are expected to occur. These environmentally mediated changes are also important when comparison is made in the assessment to the dose response exhibited by either a sufficiently similar whole mixture (e.g., comparison of the dose response of the commercial mixture that has been toxicologically tested to that of the environmental mixture) or mixture components. The concept of *sufficient similarity* is not discussed in the 1986 mixtures guidelines (U.S. EPA, 1986, 1987) (Appendix A). Common sense dictates that *sufficient similarity* entails the assumption that the toxicologic consequences of exposure to the two mixtures (i.e., the mixture of concern and the mixture on which data are available) will be identical or at least indistinguishable from one another. In practice, some degree of chemical similarity or at least an understanding of how chemical differences between the mixtures affect

toxicological activity is required. The acceptability of a surrogate, given the degree of accuracy desired in the risk assessment, should be identified in the analysis.

When the effects of such environmental processes cannot be directly measured or modeled on the mixture of interest, there is potential for substantial error in the risk assessment. The risk assessment can sometimes be modified by knowledge of the process that is generating the mixture exposure, or by information on the original mixture chemicals along with the geochemical and biochemical processes operating during their transport and over time. The degree to which environmental fate alters the exposure or the dose response changes a basic assumption of risk assessment of chemical mixtures, that of sufficient similarity. Under some circumstances, sufficiency of similarity may be gauged by the gradient of costs (monetary or environmental) of misjudging similarity, although such analyses will not be discussed here.

Whenever the mixture risk assessment is based on chemical component information and the mixture composition cannot be fully identified, the uncertainty and possible bias in the resulting risk assessment should be clearly described. Attention should also be given to the persistence of the mixture in the environment as well as to the variability of the mixture composition over time or from different sources of emissions. The assessment should also discuss methods for improving the assessment, including gathering of more data as well as employing other measurement or extrapolation techniques.

2.4.1.1. *Transport Through an Environmental Compartment*

Transport of a chemical mixture through the environmental compartments of air, soil, and water will depend upon the physical and chemical properties of the individual components or the unique properties of the chemical mixture (e.g., nonaqueous-phase liquids [NAPLs]) and the environmental medium. There are a number of examples of changes in composition or quantity of a chemical mixture as a result of environmental fate. The changes in the quantities and concentrations of chemical disinfectant by-products (occuring in chemically disinfected drinking water over time) during transport through the drinking water distribution system provide an example of the changes that can occur to a mixture during transport through an environmental compartment.

2.4.1.2. *Intercompartmental Transfer Between Environmental Compartments*

All components of a chemical mixture may not be transferred between environmental compartments at the same rate. Once released to the environment, a mixture of chemicals may be partitioned on the basis of the physical/chemical properties of each component of the mixture and the condition of the microenvironment into which the components are partitioned.

Selective movement of components can occur primarily during transport between soil, air, or water environments. For example, volatilization from the soil surface compartment to the atmospheric compartment could be important initially for the more volatile compounds in the mixture. Volatilization from dry soil surfaces is dependent on both the vapor pressure (more volatile compounds will volatilize more readily) and the ability of a compound to adsorb to soil. Volatilization from moist soil surfaces is driven by the Henry's Law constant at steady state (volatilization increases with a larger Henry's Law constant) and, as with dry soil surfaces, the ability of a compound to adsorb to the soil. Because the Henry's Law constant is defined as the ratio of a compound in air to that in water, compounds with either a high vapor pressure or compounds that have a low vapor pressure together with a low water solubility may volatilize from both moist soil and water surfaces. The rate at which a compound can volatilize from the soil surface may be attenuated if that compound is also able to adsorb strongly to soil particles. Compounds that adsorb strongly to the soil may also be physically entrained in the air as dust or moved to aquatic environments via sediment runoff. Compounds that do not adsorb strongly to the soil may leach readily through the soil column to groundwater systems if processes such as volatilization and biodegradation do not occur rapidly enough. (There are exceptions, such as where some vapor-phase pollutants in stack emissions adsorb to particulates.) The extent of soil adsorption is generally based on the organic content of the soil, although some compounds (those with a positive charge) can also adsorb to clays. A soil adsorption coefficient is defined in terms of the soil organic carbon and can be used to estimate the ability of a particular compound to leach into the soil column. The more volatile components of a chemical mixture in soil may volatilize over a several-year period and no longer be present. A risk assessment based only on the original mixture composition could then overestimate the long-term risk if the volatile chemicals were the primary toxicants. Adjustments based on other factors such as exponential decay models calibrated for the soil composition being assessed might improve the risk estimate.

The analyst should also consider differential transfer of chemicals comprising a mixture between abiotic and biotic compartments and between two different biotic compartments. For example, certain dioxin congeners released from the stacks of combustion sources appear to be selectively taken up and retained in plant tissues (Lorber et al., 1996; 1998). The relative proportions of dioxin congeners in the mixture to which humans and grazing animals are exposed through the consumption of these contaminated plants vary considerably from the original congener mixture released to the environment. The proportions of dioxin congeners in human exposures that result from consumption of the tissues of the grazing animals (e.g., beef cattle) will differ from the proportions released from the stack as well as those in the contaminated plants.

2.4.1.3. *Transformation of a Chemical Mixture or Individual Compound Into Degradation Products*

In the environment, chemical mixtures may arise or change as a result of transformation. If the various compound/s are susceptible to degradation via photolysis, hydrolysis, or biodegradation (both aerobic and anaerobic), both alteration of the profile of the original compounds in the mixture and changes in the quantity of the mixture present are possible. The processes acting to change the profile of a mixture may be affected by the point of release of the mixture (i.e., the profile from a mixture directly released to a lake may be different from that from the same mixture following long-range atmospheric transport). Transformation reactions that may differentially affect mixtures components in air, soil, and water are presented below, followed by an example using the transformation of toxaphene.

- Atmosphere: Compounds can be transformed by direct photolysis, if the compound is able to absorb light in the visible region of the spectrum, and/or by reaction with reactive photochemically generated hydroxyl radicals, nitrate radicals, and ozone (Atkinson, 1994). Reaction with hydroxyl radicals is expected to be the major degradation process in the troposphere for most molecules, and the rate of this reaction depends primarily on the chemical structure (Atkinson, 1994). Unsaturated compounds also are expected to react quickly with nitrate radicals and ozone.

- Soil: Compounds can be transformed through aerobic and anaerobic biodegradation at the soil surface. Aerobic biodegradation is controlled by concentrations of oxygen and nutrients; compounds susceptible to anaerobic biodegradation may be transformed in anaerobic microsites, which may be found within the soil column and when the soil is flooded.

- Water: Susceptible compounds may be transformed through hydrolysis (e.g., structures such as amides, alkyl halides, carbamates, and phosphoric acid esters [Lyman et al., 1990] are particularly vulnerable), direct photolysis at the water surface, and aerobic biodegradation.

The assessment of environmentally degraded or “weathered” toxaphene, previously the most heavily used pesticide in the United States, exemplifies the concerns of transformation as well as other environmental fate processes when developing a chemical mixtures risk assessment. Toxaphene is a formulation of multiple ingredients. The relative amounts of these components and their character change after toxaphene is released to the environment and the original components of the mixture are exposed to differential partitioning and transformation processes in air, water, and soil environments (U.S. EPA, 1997b).

- Toxaphene congeners are generally biologically degraded under anaerobic conditions through reductive dechlorination. Anaerobic degradation rates in soils and sediments are expected to be determined largely by qualities of the original component molecules and the environment's potential to interact and change the molecules' structure (Fingerling et al., 1996; Smith and Willis, 1978). The stability of reaction products, whether in soil or sediment, seems to depend on the position of the various chlorine atoms.
- Under aerobic conditions toxaphene degrades slowly, if at all (Parr and Smith, 1976; Bidleman et al., 1981; Mirsatari et al., 1987; Nash and Woolson, 1967).
- In general, the lower chlorinated toxaphene congeners are more easily vaporized than are the higher chlorinated congeners (Seiber et al. [1979] showed soil surface enrichment of the less volatile, more chlorinated compounds through GC analysis); however, both can be atmospherically transported.
- Toxaphene, particularily the more volatile components, may be transported far from the initial source by long-range atmospheric transport processes.
- Once deposited in water, the higher chlorinated congeners can bioaccumulate in the food chain because of their lipophilicity.

The composition of "weathered" toxaphene samples may be different, depending on the environmental processes to which the original agent was exposed. For example, toxaphene extracted from an anaerobic soil does not resemble that from an aerobic soil, and toxaphene detected in an air sample from the Arctic does not resemble the toxaphene residue obtained from the blubber of an Arctic seal. Site-specific consideration of the partitioning and transformation processes is needed for different environments. The resulting changes in chemical composition of the original mixture over time will affect the toxicity of the mixture.

For another example, when the primary change to a mixture is believed to be the degree of halogenation or other substitution, some adjustment of the estimated exposure or toxic potency may be possible. One example (discussed in Section 3.4) concerns combinations of PCBs, for which EPA has developed specific methodology to alter the toxic potency on the basis of site-specific environmental factors.

2.4.2. Importance of the Exposure Sequence for Multiple Chemicals

The order in which chemical exposures occur and the time between exposures to different chemical agents may affect the nature of the response to the chemical insult. For example, the sequence or pattern of exposure is important for compounds that have been described as initiators and those described as promoters of carcinogenicity. There is evidence to suggest that exposure to certain compounds results in an irreversible change in the affected cells and progeny (the cell is said to be initiated). When the initial exposure is followed by repeated doses of a second chemical agent (i.e., the promoter), tumors occur. In the absence of either the initiator or the

promoter, or if the order is reversed, tumors do not occur. An example of an initiator-promoter sequence is the application of a PAH (initiator) (e.g., benzo[b]fluoranthene) followed by repeated applications of 12-o-tetradecanoyl phorbol-13-acetate (TPA) to the skin of shaved mice (Amin et al., 1985).

2.4.3. Routes of Exposure

In environmental health risk assessments, analysts typically consider three routes of human exposure: oral, dermal, and inhalation. Differences in the properties of the cells that line the surfaces of the gastrointestinal tract, the skin, and the air pathways and lungs may result in different intake patterns of chemical mixture components depending on the route of exposure. Additionally, chemicals in a mixture may partition to contact media differently, resulting in different potential routes of exposure (see Section 2.4.1). In chemical mixtures risk assessment, the issue becomes how and when to combine routes. EPA is still developing approaches for this. EPA (1998c) recommends that route-to-route conversion should be attempted only for dermal exposures at this time. Adequate inhalation-to-oral conversion methods for steady-state conditions have not yet been developed. A general outline of the oral-to-inhalation extrapolation process and a discussion of route-to-route extrapolation issues can be found in Gerrity and Henry (1990) and in EPA's Reference Concentration methodology document (U.S. EPA, 1994a). Until such methodology is developed, inhalation and oral risk characterization should be carried out separately. The assessor should note, however, that total risk from the mixture could be underestimated by not combining all routes of exposure, because the total exposure is not characterized and the chemical interactions may not be considered.

Multiple-route exposures can be combined in two different ways: summing the absorbed daily doses or summing the (external) oral equivalent daily doses. Both approaches require an estimate for the oral absorption fraction, but the latter is adopted here as it is simpler for consideration with standard toxicity comparison values based on ingestion (e.g., RfD).

A number of factors might contribute to differences in toxicologic effectiveness between oral and dermal exposures at equal dosages. The most obvious relates to differences in absorption rates between the two routes. Other potential contributing factors include differing sensitivity of absorption sites to damage and differences in toxicokinetics (i.e., distribution, metabolism, elimination) between exposure routes. Ideally, the conversion from dermal to equivalent oral dose would be based on experimentally derived values that characterize the relationship between the doses that produce a particular toxicity by each of the different routes. In practice, however, the conversion usually will be based on absorption factors because of a general absence of appropriate data.

2.4.4. Exposure Assessment Summary

This section summarizes a few important concepts related to chemical mixtures exposure assessment. Once a chemical mixture is released to the environment, its concentration and composition may change through partitioning into abiotic and biotic compartments and through transformation mediated by the environment and biota. The physical/chemical properties of each component of the mixture (or the properties of the mixture as a whole) and the condition of the microenvironment into which the components are partitioned may change the magnitude and the routes of human exposure. Partitioning and transformation of the mixture components will affect the routes of exposure. Ideally, chemical mixture exposures through different routes can be integrated through measurement data or a validated physiologically based pharmacokinetic (PBPK) model; at this time, approaches are still evolving, particularly for combining inhalation and oral exposures. The sequence of exposures to different chemical agents is clearly important for some responses. A number of other issues will be deferred for later discussion by the Agency; these include chemical mixtures with intrinsically unique properties (e.g., NAPLs), mass balance within chemical mixtures assessments, assessing risk of unidentified components of chemical mixtures, measurement issues, and component bioavailability.

2.5. DATA AVAILABLE ON WHOLE MIXTURES

Whenever possible, the preferred approach to the health risk evaluation of chemical mixtures is to perform the assessment using health effects and exposure data on the whole mixture. Such data include human epidemiologic, clinical, or occupational studies; animal studies on the complex mixture; or in vitro data on the complex mixture. Figure 2-1 shows that the whole-mixtures data can then be divided into subsets of data directly on the mixture of concern, data on a sufficiently similar mixture, or data on a group of similar mixtures. This guidance document discusses these situations and offers some examples of how to approach a whole-mixture health risk assessment.

2.5.1. Data Available on the Mixture of Concern

Exposure and toxicity data directly on the mixture of concern are most likely to be available for highly complex mixtures, such as coke oven emissions, which are generated in large quantities and associated with or suspected of causing adverse health effects. The evaluation of such a mixture requires scientific judgment regarding the stability of the mixture in the environment and the linkage of the observed human health effect to the mixture exposure. Toxicity data obtained from concentrates or extracts of the original mixture of concern may not be predictive of human toxicity to the original mixture. Such data are more properly handled using procedures developed for toxicologically similar mixtures (Sections 2.5.3, 3.3).

2.5.1.1. *User Fact Sheet: Mixture of Concern RfD/C or Slope Factor*

The user of this guidance document can use Figure 2-1 to determine if data are available directly on the mixture of concern. Then a procedure is suggested for estimating either a cancer slope factor or a reference dose/concentration (RfD/C), as encapsulated in the following user-information fact sheet.

Approach:	Mixture of Concern RfD/C or Slope Factor
Type of Assessment:	Dose-Response Toxicity Value
Section(s):	3.1, 3.2
References:	Examples can be found on IRIS (U.S. EPA, 2000a).
Data Requirements:	Toxicity data are available on the mixture of concern. Examples of such data are human epidemiologic data from an occupational setting, human data from a clinical study, or animal toxicology data on the complex mixture.
Strategy of Method:	Estimate dose-response toxicity value directly from data on complex mixture of concern, using the same procedures as those used for single chemicals.
Ease of Use:	Calculations are simple.
Assumptions:	Composition of the test mixture is functionally the same as what is found in the environment. Test data are adequate to account for all sensitive endpoints.
Limitations:	Data are rarely available.
Uncertainties:	Scientific judgments of the chemical composition of the mixture; toxicologic relevance of the laboratory data to the environmental mixture.

2.5.2. Data Available on a Sufficiently Similar Mixture

If data are not available on the mixture of concern, the risk assessment may be based on data on a sufficiently similar mixture. A mixture is sufficiently similar to the mixture of concern when its components are not very different and are contained in about the same proportions as the mixture of concern. In addition, if information exists on differences in environmental fate, uptake and pharmacokinetics, bioavailability, or toxicologic effects for either of these mixtures or their components, it should be considered in the determination of sufficient similarity. If such data are available, an attempt should be made to determine if significant and systematic differences exist between the chemical mixtures. If no significant differences are noted, then a risk assessment may be performed using data on the sufficiently similar mixture as a surrogate for the mixture of concern.

2.5.2.1. *User Fact Sheet: Sufficiently Similar Mixture RfD/C or Slope Factor*

The user of this guidance document can use Figure 2-1 to determine that the data available are on a mixture that is sufficiently similar to the mixture of concern. Then a procedure is suggested for estimating either a cancer slope factor or a reference dose/concentration (RfD/C), as encapsulated in the following user-information fact sheet.

Approach:	Sufficiently Similar Mixture RfD/C or Slope Factor
Type of Assessment:	Dose-Response Toxicity Value
Section(s):	3.1, 3.2
References:	New procedure.
Data Requirements:	Toxicity data are available on a mixture that is judged as sufficiently similar to the mixture of concern in the environment. No data are available on the mixture of concern. Examples of such data are human epidemiologic data from an occupational setting, human data from a clinical study, or animal toxicology data on the complex mixture.
Strategy of Method:	Estimate dose-response toxicity value using data on the sufficiently similar mixture as a surrogate for data on the mixture of concern, using the same procedures as those used for single chemicals.
Ease of Use:	Calculations are simple.
Assumptions:	Composition of the sufficiently similar mixture is functionally the same as what is found in the environment. Test data are adequate to account for all sensitive endpoints. Similarity judgment across the mixtures must be made and supported.
Limitations:	Availability of data is limited.
Uncertainties:	Scientific judgments of sufficient similarity, chemical composition and stability of the two mixtures; toxicologic relevance of the laboratory data to the environmental mixture.

2.5.3. Data Available on a Group of Similar Mixtures

In some cases, data are available on a group of similar mixtures that are known to be generated by the same commercial process or emissions source but that vary slightly in composition depending on factors such as time since emission, environmental transformation, or geographic location of emission sources. Data are then available on several mixtures with approximately the same components but with slightly different component exposure levels, so that the likely range of compositional variation is covered. Thus, risk assessors can use toxicity and exposure data that exist on the group of similar mixtures and extrapolate in order to perform a risk assessment on the less well-studied or environmentally transformed mixtures that belong to that same group.

2.5.3.1. *User Fact Sheet: Comparative Potency*

The user of this guidance document can use Figure 2-1 to determine that the data available are on a group of similar mixtures. Then a procedure is suggested for using a comparative potency approach to estimating a cancer slope factor, as encapsulated in the following user-information fact sheet.

Approach:	Comparative Potency
Type of Assessment:	Dose-Response Toxicity Values for Cancer, Genetic Toxicity
Section(s):	3.1, 3.3
References:	Used for combustion mixtures (Lewtas, 1985, 1988; Nesnow, 1990).
Data Requirements:	Method requires short-term data on several similar mixtures including the mixture of concern, and at least one data point from a chronic in vivo study on one of these mixtures. Examples of such data are in vitro mutagenicity assays and chronic rodent bioassays.
Strategy of Method:	Estimate dose-response value using relationships across similar mixtures and similar assays to extrapolate to a value for the mixture of concern.
Ease of Use:	Calculations involve some statistical modeling and toxicologic judgment. Method is data intensive with short-term assay data required.
Assumptions:	Assumes the potency change for similar mixtures across assays is the same for all similar mixtures. Test data are adequate to account for all sensitive endpoints. Similarity judgment across the mixtures must be made and supported.
Limitations:	Availability of data is limited.
Uncertainties:	Scientific judgments of sufficient similarity relative to chemical composition and toxicologic activity of the mixtures.

2.5.3.2. *User Fact Sheet: Geographic Site-Specific Assessments*

The user of this guidance document can follow Figure 2-1 to determine that the data available are on a group of similar mixtures. Then a procedure is suggested for estimating risk from exposure to the mixture by using a geographic site-specific assessment, as detailed in the following user-information fact sheet.

Approach:	Geographic Site-Specific Assessment
Type of Assessment:	Risk Characterization for Any Toxic Endpoint
Section(s):	3.1, 3.4
References:	Used for cancer assessment of PCBs (U.S. EPA, 1996c)
Data Requirements:	Method requires both toxicity and exposure data on the mixture's components.
Strategy of Method:	Toxicity data on the commercial mixture are used to estimate a range of toxicity values that are then adjusted for alterations in the mixture's composition because of environmental factors to produce a risk estimate for the total mixture.
Ease of Use:	Complicated to use. Data intensive.
Assumptions:	Requires the user to make assumptions about the fate and transport of groups of chemicals.
Limitations:	Some data restricted by similarity. Restricted to specific conditions. Limited by data quality.
Uncertainties:	Scientific judgment of fate and transport. Accuracy of exposure data.

2.6. DATA AVAILABLE ON MIXTURE COMPONENTS

If data are not available on an identical or reasonably similar mixture, the risk assessment may be based on the toxic or carcinogenic properties of the components in the mixture. When quantitative information on toxicologic interaction exists, even if only on chemical pairs, it should be incorporated into the component-based approach. When there is no adequate interactions information, dose- or risk-additive models are recommended. The primary criterion for choosing between dose addition and response addition is the toxicologic similarity among the chemicals in the mixture. This decision should be based on information about the toxicologic and physiologic processes involved, the single-chemical dose-response relationships, and the type of response data available. The risk assessment using component data should then begin with selection of the most appropriate concept for the chemicals in the mixture.

2.6.1. Toxicologic Similarity and Dose Addition

In the simplest terms, chemicals can be considered as dose additive if each chemical can be thought of as a concentration or dilution of every other chemical in the mixture. The chemicals are assumed to behave similarly in terms of the primary physiologic processes (uptake, metabolism, distribution, elimination) as well as the toxicologic processes. The mathematical description of dose addition requires a constant proportionality between the effectiveness of the two chemicals. Three component methods that are based on dose addition are discussed in this document: the HI, the Relative Potency Factor (RPF) method, and the Toxicity Equivalence Factor method, which is a special case of the RPF method. They differ in the required knowledge about toxic mechanism and in the extent over which toxicologic similarity is assumed. In each method, the exposure levels are added after being multiplied by a scaling factor that accounts for differences in toxicologic potency.

2.6.1.1. *User Fact Sheet: Hazard Index*

The user of this guidance document can follow Figure 2-1 to determine that the data available are on the components of the mixture of concern and that there is evidence of toxicologic similarity of the components. Then a procedure is suggested for estimating a Hazard Index, an indication of risk from exposure to the mixture, as encapsulated in the following user-information fact sheet.

Approach:	Hazard Index
Type of Assessment:	Risk Characterization for Any Toxic Endpoint
Section(s):	4.1, 4.2
References:	Used in Superfund site assessments (U.S. EPA, 1989a).
Data Requirements:	Method requires both toxicity and exposure data on the mixture's components. Good dose-response data are needed, such as what is available on IRIS (U.S. EPA, 2000a).
Strategy of Method:	Scale individual component exposure concentrations by a measure of relative potency (typically, divide by a Reference Dose/Concentration [RfD/C]) for components with a similar mechanism-of-action. Add scaled concentrations to get an indicator of risk from exposure to the mixture of concern.
Ease of Use:	Easy to calculate.
Assumptions:	Applies dose addition, which carries with it assumptions of same mode of action and similarly shaped dose-response curves across the components. The "common mode-of-action" assumption can be met by using a surrogate of same target organ.
Limitations:	Exposure data should be at relatively low levels (near no-adverse-effect levels) at which interaction effects are not expected. RfD/C values across components vary in their uncertainty, so other measures of potency may be more appropriate.
Uncertainties:	Similarity of mechanism-of-action. Accuracy of exposure data.

2.6.1.2. *User Fact Sheet: Relative Potency Factors*

The user of this guidance document can follow Figure 2-1 to determine that the data available are on the components of the mixture of concern and that there is evidence of toxicologic similarity of the components. Then a procedure is suggested for estimating risk from exposure to the mixture by using Relative Potency Factors, as encapsulated in the following user-information fact sheet.

Approach:	Relative Potency Factors
Type of Assessment:	Dose-Response Assessment for Any Toxic Endpoint
Section(s):	4.1, 4.4
References:	New Procedure
Data Requirements:	Method requires both toxicity and exposure data on the mixture's components. Toxicity data are missing for some components.
Strategy of Method:	Scale component exposure concentrations relative to potency of an index chemical (typically the best-studied component) following expert committee consensus. Add scaled concentrations. Use dose-response curve of index chemical to generate response estimate for sum of scaled concentrations.
Ease of Use:	Complicated to use. Requires some statistical modeling and judgment of relative potency factors.
Assumptions:	Based on dose addition which carries with it assumptions of same mode of action and similarly shaped dose-response curves across the components. The "common mode-of-action" assumption can be met using a surrogate of toxicologic similarity, but for specific conditions (endpoint, route, duration).
Limitations:	Limited by data quality and similarity. May not have data from all routes of exposure of interest. Same mode-of-action across components may not be known.
Uncertainties:	Judgment of relative potency factors. Similarity of toxicologic action. Missing data on some components.

2.6.2. Independence and Response Addition

Response addition may apply when components act on different systems or produce effects that do not influence each other. Under response addition, the chemicals in the mixture are assumed to behave independently of one another, so that the body's response to the first chemical is the same whether or not the second chemical is present. Mathematically, response addition can be described by the statistical law of independent events, with "response" measured by the percentage of exposed animals that show toxicity or the proportion of the population responding. Response addition is particularly useful when the effects of concern are thought to be present at low dose levels for each of the component chemicals, even though it is highly unlikely the effects are capable of being observed at these low levels in the environment. When interaction data are available on any of the components in the mixture, the risk assessor may provide a qualitative discussion of the likely effect of these data on the outcome of the mixture risk assessment under response addition (see Sections 2.2.4, 4.5.4).

2.6.2.1. *User Fact Sheet: Response Addition*

The user of this guidance document can follow Figure 2-1 to determine that the data available are on the components of the mixture of concern and that there is evidence of toxicologic independence of action. Then a procedure is suggested for estimating risk from exposure to the mixture by using Response Addition, as encapsulated in the following user information fact sheet.

Approach:	Response Addition
Type of Assessment:	Risk Characterization for Any Toxic Endpoint
Section(s):	4.1, 4.5
References:	Used extensively for cancer. Used in Superfund site assessments (U.S. EPA, 1989a).
Data Requirements:	Method requires both toxicity data (measured in percent responding) and exposure data on the mixture's components. Good dose-response data are needed, such as what is available on IRIS (U.S. EPA, 2000a).
Strategy of Method:	Risk of an effect is estimated for each component using its dose-response curve at the component's exposure concentration. Component risks are added, using the independence formula, to yield a risk estimate for the total mixture for the specific exposure.
Ease of Use:	Easy to calculate.
Assumptions:	Assumes toxicologic independence of action. Assumes interactions are not significant at low exposures.
Limitations:	Limited to low exposure concentrations. Slight overestimate of mixture's upper bound on risk when adding individual component upper bound estimates. Restricted to independence of action.
Uncertainties:	Independence of action. Accuracy of exposure data. Individual risk estimates may vary in quality.

2.6.3. Interactions Data

Toxicologic interactions are operationally defined by the existence of data showing significant deviations from a "no interaction" prediction; that is, the response is different from what would be expected under an assumption of additivity (e.g., dose-additive, response-additive). Types of interactions among mixture components that can affect toxicologic response to the whole mixture include chemical-to-chemical, toxicokinetic, and toxicodynamic interactions (see Table B-2 and Appendix C). The impact of these constituent interactions on toxicologic response can be less than additive (e.g., antagonistic) or greater than additive (e.g., synergistic). The component-based method discussed in this document that incorporates interactions information is the interaction-based HI.

2.6.3.1. *User Fact Sheet: Interaction-Based Hazard Index*

The user of this guidance document can follow Figure 2-1 to determine that the data available are on the components of the mixture of concern and that interactions data are available. Then a procedure is suggested for estimating risk from exposure to the mixture by incorporating information on binary combinations of the components using an interaction-based hazard index, as encapsulated in the following user information fact sheet.

Approach:	Interaction-Based Hazard Index
Type of Assessment:	Risk Characterization for Any Toxic Endpoint
Section(s):	4.1, 4.3
References:	New procedure (Hertzberg et al., 1999).
Data Requirements:	Method requires both toxicity and exposure data on the mixture's components, and interactions data on at least one pair of components.
Strategy of Method:	Scale component exposure concentrations by a measure of relative potency (typically, divide by a reference dose/concentration [RfD/C]) for components with a similar mechanism-of-action. Modify this term with data on binary interactions. Add scaled/modified concentrations to provide an indicator of risk from exposure to the mixture of concern.
Ease of Use:	Complicated to use.
Assumptions:	Assumes binary interactions are the most important. Assumes interaction magnitude is not dose dependent, but depends on component proportions.
Limitations:	Limited interactions data are available. Model with relative proportions is untested. Interaction magnitude is often a default because of lack of measurement data.
Uncertainties:	Binary interactions used to represent the interactions for the whole mixture. Accuracy of exposure data. Accuracy of default for interaction magnitude.

2.7. FUTURE DIRECTIONS

2.7.1. Overview

Risk assessment methods for chemical mixtures are progressing along paths similar to risk assessment for single chemicals, by incorporating more knowledge of specific modes of toxicologic action of the chemicals and by greater use of statistical methods and mathematical models. Where the field differs, however, is in the more extensive use of quantitative inference from tested chemicals to untested chemicals. Mixture exposures can be extremely varied, with differences in total dose, composition, and relative proportions. Consequently, only a small fraction of environmental mixtures can actually be tested for dose-response characteristics. Two options then seem feasible: directly investigating a few high-priority mixtures, and, for the remainder, developing extrapolation methods for using available data on the mixture components or on similar mixtures.

The first option requires priority setting, which for mixtures is its own research area (Cassee et al., 1998). The characteristics to include in a mixture prioritization scheme should parallel those often cited for single chemicals: target

those mixtures posing the highest public health risk. The supporting data could include annual emissions of mixtures, frequency of occurrence of mixtures in the environment, identity of mixtures containing highly toxic chemicals, or documented health problems in populations exposed to identified mixtures. Because most interaction data are on chemical pairs, one approach would include the frequency of occurrence of chemical pairs in the media associated with the exposure scenario to be regulated. The prioritization should also consider the availability of interaction data. For high-priority mixtures lacking such data, other assessment methods may be needed. The various regulatory program areas, such as Superfund waste sites, ambient air, and drinking water, pose substantially different kinds of mixtures and exposure conditions, so that a priority list for one program may not be appropriate for a different regulatory program.

Once a few mixtures posing the highest concern have been identified, researchers should seek to evaluate their exposure, toxicity, and risk characteristics. Because even the highest priority mixtures are likely to pose complex and varied exposure possibilities, much of the research effort should involve developing highly efficient experimental designs, short-term toxicity assays, and uncertainty methods so that several scenarios can be characterized for each mixture.

The second option, for addressing all the remaining mixtures, is to develop methods that can extrapolate exposure and toxicity estimates from available data to the scenario of concern. In addition to the issues being addressed by extrapolation methods for single chemicals (e.g., cross-species, cross-route), mixtures issues also include interactions and changes in composition. Interactions issues include the commonly observed toxicologic interactions that influence pharmacokinetics, as well as the less-studied areas of physiological interactions between affected tissues or organs, and the biochemical and physical interactions affecting degradation and transport of mixtures in environmental media. Because of the wide variety of mixture exposures, all relevant information should be tapped to improve the understanding of the basic biological and chemical processes. For example, to improve dose-response extrapolation, toxicology experiments, epidemiology and occupational studies, and mathematical model development should be pursued simultaneously.

Mixtures research should be efficient. The complexity of the issues is beyond the reach of any single agency. Sharing of resources and information within different sectors of EPA as well as with other agencies is essential. Several such efforts are underway. The Integral Search System (Arcos et al., 1988) and the Mixtox database (Marnicio et al., 1991) are two EPA collections of bibliographic summaries of interaction studies that are available to the public. Additional databases should be developed, perhaps jointly with the public, on mechanisms and modes of toxicologic interaction and on mathematical models of biological processes influencing

the interactions. The National Institute for Occupational Safety and Health (NIOSH) has a Mixed Exposures research program whose advisory committee includes representatives from EPA, other federal agencies, and research institutions. EPA, NIOSH, and the Agency for Toxic Substances Disease Registry (ATSDR) have organized the Mixed Exposures Research Group (MERG), composed of almost 20 federal and state agencies, to share regulatory approaches. MERG seeks to facilitate interagency communication and jointly sponsored research projects on mixtures. Additional cooperative efforts should be pursued with the public and foreign agencies.

Mixture risk assessment methods should ideally be developed in conjunction with those laboratory and field studies that are needed for implementation as well as validation. Otherwise, the methods become conceptual models that cannot feasibly be applied, or decision tools whose accuracy cannot be tested. One example concerns interaction studies, such as those detailed in the EPA's Mixtox database (Marnicio et al., 1991; U.S. EPA, 1990) of in vivo toxicologic interaction studies. In the Mixtox database, 95% of the studies involve only pairs of chemicals (Teuschler and Hertzberg, 1995). Consequently, the interaction-based Hazard Index (Section 4.3) was developed for pairwise interactions to allow use of available data. Interaction studies are in progress by research groups in EPA's National Center for Environmental Assessment (NCEA) and National Health and Environmental Effects Research Laboratory (NHEERL) to provide the toxicity data and data analysis methods for validation of the index.

The information required for evaluation of the extrapolation methods in this document is generally not yet available. The number of pairs studied for interactions is a small fraction of the number of possible chemical combinations, and the number of whole mixtures studied is far smaller yet. For example, with a simple mixture of only 20 chemicals, there are 190 pairs, but over a million possible combinations (pairs, triples, etc.). Because of this sparseness of existing data, both on whole mixtures and on interactions, the accuracy of these extrapolation methods will be difficult to judge. The inferential procedures for mixture risk discussed in this document are then likely to be adopted based on scientific plausibility and on relatively few validation studies. The validation process is valuable, even when incomplete. As was found with the analysis of the consistency of pairwise interactions (Durkin et al., 1995), the evaluation of the mixture risk tools will likely spawn research questions that lead to new statistical, exposure, and toxicologic studies, and subsequently to better risk tools.

2.7.2. Research Suggestions for Improving Mixture Risk Assessment

Several research directions have been suggested during the development of this guidance document. Although specific projects have been identified related to dose-response assessment, the highest priority was the preparation of guidance on exposure assessment of mixtures. Some of the key concerns with exposure assessment are discussed in this document (Section 2.4). The

need is for specific procedures for measurement and modeling of exposures for various scenarios, along with the corresponding methods for characterizing the uncertainties. The Risk Assessment Forum created an advisory panel in 1999 to decide the scope and project requirements for a framework for cumulative risk assessment. A major component of that framework is the exposure assessment of mixtures. Some specific areas for exposure assessment that have been suggested during review of this guidance are given in the list below.

Among the next highest priorities was research aimed at the evaluation and improvement of the dose-response methods in this guidance document. In particular, the comparative potency method for whole mixtures and the interaction-based Hazard Index need to be demonstrated with different kinds of mixtures. Methods for validation of these two methods also need to be developed, followed by the validation exercise itself for several different mixtures.

The most often mentioned research area was uncertainty analysis. Each of the methods in this guidance document produces a single risk estimate. An initial goal is to present that risk estimate as a plausible range in addition to the single recommended value. A related goal is to present a range of risk estimates that reflects all the risk methods applied to the mixture of concern, i.e., the uncertainty in model selection. Data uncertainties should also be addressed, at least by sensitivity analysis. Subsequent efforts should pursue more complete uncertainty characterization, including methods for choosing the default distributions for the parameters and variables in each method. Uncertainty characterization is also one of the components of the Forum's cumulative risk framework project, so further work will commence in this area over the next few years.

The other main research needs raised during the authoring and review of this guidance document covered a wide range of scientific areas. The most commonly discussed topics are in the following list. The research areas are roughly grouped by scientific discipline or application.

<u>Exposure assessment</u>

- data and models for degradation over several years (e.g., pathogens in groundwater, pesticide mixtures in soil).
- models/data for chemical and biological interactions influencing mixture transport.
- mixture changes (chemical composition, relative proportions) from facility failures (e.g., drinking water, municipal combustors).
- procedures for artificial degradation or weathering of complex mixtures.
- procedures for monitoring mixtures when there are hot spots with each spot having a different driver chemical.
- biomarkers of exposure that are specific to single chemicals or chemical classes and mathematical models that relate the biomarker to existing or prior external exposure levels, and to tissue levels and/or tissue-specific toxic effects.

Statistical/mathematical methods

- formulas for incorporating independence when adding upper-bound risks ($n > 3$).
- concepts and methods for tolerance distributions for $n > 2$ chemicals.
- uncertainty analysis, i.e., Bayesian, Monte Carlo simulation for each of the mixture risk assessment procedures.
- efficient and stable numerical methods for modeling highly complex interacting systems (hundreds of chemicals, multiple tissues, time-variable exposures).
- statistical graphics methods for demonstrating and displaying interactions in multichemical mixtures ($n > 5$).

Biomathematical models

- models for describing the dependence of interaction magnitude on total dose and on component fractions.
- biologically based models that separate out the relative differences of chemicals in terms of pharmacokinetics and pharmacodynamics.
- models that incorporate aging and growth, and more physiological processes and factors than just flows to major organs and tissues.
- models for initiation-promotion interactions that include background exposures to initiators or promoters.

Human studies

- database of epidemiology studies with exposure-response information on mixtures.
- database of occupational health studies with exposure-response information on mixtures.
- methods for estimating interaction magnitudes in epidemiology studies that relate to (are consistent with) physiologic measures of interaction magnitude.
- information on background exposure levels, background prevalence of health conditions, and those population characteristics that indicate increased susceptibility to toxic chemicals, including models that quantify the influence of population characteristics on toxicology.

Toxicology

- modes and mechanisms of interaction for carcinogens.
- data describing the dependence of interaction magnitude on total mixture dose and on component fractions.
- concordance across animal species of specific toxic effects, modes of action, and modes of interaction.
- data and modes of interaction for inhibition (one chemical is nontoxic).
- data and concepts for particulate interactions with other airborne chemicals.

- more examples and methods for short-term whole-mixture toxicity testing, particularly data showing the representativeness of in vitro studies to represent in vivo toxicity.
- relationships between mode of toxic action and mode of interaction.
- concepts, mechanisms or modes of action, or toxicity data to explain the mathematical interaction models of proportional response addition and straight-line isoboles that are not parallel.
- interaction studies on major chemical classes to establish empirical interaction classes based on interaction patterns.
- test procedures that mimic real-world exposures (e.g., species-adjusted intermittent exposures to correspond to occupational exposure patterns)
- biomarkers of toxicity that are specific to single (or related) toxic effects and mathematical models that relate the biomarker to actual measurable toxic endpoints.

<u>Risk methods</u>

- development of screening assays for mixtures to identify combinations of chemicals that are most toxic or that potentially interact.
- risk estimation for a mixture of mixed types, including similar, independent, and interacting chemicals with same target organ, e.g., for classes with similar (RPF) chemicals and other chemicals.
- risk estimates or qualitative risk indicators for unidentified chemicals in a mixture (see U.S. EPA, 1998d. Comparative risk framework methodology and case study. SAB external review draft. NCEA-C-0135).
- MOE methods for carcinogens using response addition.
- RPFs from dose-response data on all chemicals, as improvement over HI because it allows actual estimate of toxicity from the index chemical's dose-response curve.
- use of interaction patterns for estimating interaction direction in a chemical class.
- methods for prioritizing chemical pairs (air, drinking water) for further study on the basis of health risk.
- methods for prioritizing complex mixtures for further study on the basis of health risk.
- methods for prioritizing complex mixtures for further study on the basis of degradation potential.

EPA/630/R-98/002
September 1986

APPENDIX A

Guidelines for the Health Risk Assessment of Chemical Mixtures

Published on September 24, 1986, Federal Register 51(185):34014-34025

Risk Assessment Forum
U.S. Environmental Protection Agency
Washington, DC

DISCLAIMER

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Note: This document represents the final guidelines. A number of editorial corrections have been made during conversion and subsequent proofreading to ensure the accuracy of this publication.

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GUIDELINES FOR THE HEALTH RISK ASSESSMENT OF CHEMICAL MIXTURES [FRL-2984-2]

AGENCY: U.S. Environmental Protection Agency (EPA).

ACTION: Final Guidelines for the Health Risk Assessment of Chemical Mixtures.

SUMMARY: The U.S. Environmental Protection Agency is today issuing five guidelines for assessing the health risks of environmental pollutants. These are:

Guidelines for Carcinogen Risk Assessment

Guidelines for Estimating Exposures

Guidelines for Mutagenicity Risk Assessment

Guidelines for the Health Assessment of Suspect Developmental Toxicants

Guidelines for the Health Risk Assessment of Chemical Mixtures

This notice contains the Guidelines for the Health Risk Assessment of Chemical Mixtures; the other guidelines appear elsewhere in today's Federal Register.

The Guidelines for the Health Risk Assessment of Chemical Mixtures (hereafter "Guidelines") are intended to guide Agency analysis of information relating to health effects data on chemical mixtures in line with the policies and procedures established in the statutes administered by the EPA. These Guidelines were developed as part of an interoffice guidelines development program under the auspices of the Office of Health and Environmental Assessment (OHEA) in the Agency's Office of Research and Development. They reflect Agency consideration of public and Science Advisory Board (SAB) comments on the Proposed Guidelines for the Health Risk Assessment of Chemical Mixtures published January 9, 1985 (50 FR 1170).

This publication completes the first round of risk assessment guidelines development. These Guidelines will be revised, and new guidelines will be developed, as appropriate.

EFFECTIVE DATE: The Guidelines will be effective September 24, 1986.

FOR FURTHER INFORMATION CONTACT: Dr. Richard Hertzberg, Waste Management Division, U.S. Environmental Protection Agency, Atlanta Federal Center, 100 Alabama St., SW, Atlanta, GA 30303-3104, TEL: 404-562-8663.

SUPPLEMENTARY INFORMATION: In 1983, the National Academy of Sciences (NAS) published its book entitled *Risk Assessment in the Federal Government: Managing the Process.* In that book, the NAS recommended that Federal regulatory agencies establish "inference guidelines" to ensure consistency and technical quality in risk assessments and to ensure that the risk assessment process was maintained as a scientific effort separate from risk management. A task force within EPA accepted that recommendation and requested that Agency scientists begin to develop such guidelines.

General

The guidelines published today are products of a two-year Agencywide effort, which has included many scientists from the larger scientific community. These guidelines set forth principles and procedures to guide EPA scientists in the conduct of Agency risk assessments, and to inform Agency decision makers and the public about these procedures. In particular, the guidelines emphasize that risk assessments will be conducted on a case-by-case basis, giving full consideration to all relevant scientific information. This case-by-case approach means that Agency experts review the scientific information on each agent and use the most scientifically appropriate interpretation to assess risk. The guidelines also stress that this information will be fully presented in Agency risk assessment documents, and that Agency scientists will identify the strengths and weaknesses of each assessment by describing uncertainties, assumptions, and limitations, as well as the scientific basis and rationale for each assessment.

Finally, the guidelines are formulated in part to bridge gaps in risk assessment methodology and data. By identifying these gaps and the importance of the missing information to the risk assessment process, EPA wishes to encourage research and analysis that will lead to new risk assessment methods and data.

Guidelines for Health Risk Assessment of Chemical Mixtures

Work on the Guidelines for the Health Risk Assessment of Chemical Mixtures began in January 1984. Draft guidelines were developed by Agency work groups composed of expert scientists from throughout the Agency. The drafts were peer-reviewed by expert scientists in the fields of toxicology, pharmacokinetics, and statistics from universities, environmental groups, industry, labor, and other governmental agencies. They were then proposed for public comment in the Federal Register (50 FR 1170). On November 9, 1984, the Administrator directed that Agency offices use the proposed guidelines in performing risk assessments until final guidelines became available.

After the close of the public comment period, Agency staff prepared summaries of the comments, analyses of the major issues presented by the commentators, and preliminary Agency

responses to those comments. These analyses were presented to review panels of the SAB on March 4 and April 22-23, 1985, and to the Executive Committee of the SAB on April 25-26, 1985. The SAB meetings were announced in the Federal Register as follows: February 12, 1985 (50 FR 5811), and April 4, 1985 (50 FR 13420 and 13421).

In a letter to the Administrator dated June 19, 1985, the Executive Committee generally concurred on all five of the guidelines, but recommended certain revisions and requested that any revised guidelines be submitted to the appropriate SAB review panel chairman for review and concurrence on behalf of the Executive Committee. As described in the responses to comments (see Part B: Response to the Public and Science Advisory Board Comments), each guidelines document was revised, where appropriate, consistent with the SAB recommendations, and revised draft guidelines were submitted to the panel chairmen. Revised draft Guidelines for the Health Risk Assessment of Chemical Mixtures were concurred on in a letter dated August 16, 1985. Copies of the letters are available at the Public Information Reference Unit, EPA Headquarters Library, as indicated elsewhere in this notice.

Following this Preamble are two parts: Part A contains the Guidelines and Part B the Response to the Public and Science Advisory Board Comments (a summary of the major public comments, SAB comments, and Agency responses to those comments).

The SAB requested that the Agency develop a technical support document for these Guidelines. The SAB identified the need for this type of document due to the limited knowledge on interactions of chemicals in biological systems. Because of this, the SAB commented that progress in improving risk assessment will be particularly dependent upon progress in the science of interactions.

Agency staff have begun preliminary work on the technical support document and expect it to be completed by early 1987. The Agency is continuing to study the risk assessment issues raised in the guidelines and will revise these Guidelines in line with new information as appropriate.

References, supporting documents, and comments received on the proposed guidelines, as well as copies of the final guidelines, are available for inspection and copying at the Public Information Reference Unit (202-382-5926), EPA Headquarters Library, 401 M Street, SW, Washington, DC, between the hours of 8:00 a.m. and 4:30 p.m.

I certify that these Guidelines are not major rules as defined by Executive Order 12291, because they are nonbinding policy statements and have no direct effect on the regulated

community. Therefore, they will have no effect on costs or prices, and they will have no other significant adverse effects on the economy. These Guidelines were reviewed by the Office of Management and Budget under Executive Order 12291.

Dated: August 22, 1986

Signed by EPA Administrator
Lee M. Thomas

PART A: GUIDELINES FOR THE HEALTH RISK ASSESSMENT OF CHEMICAL MIXTURES

1. INTRODUCTION

The primary purpose of this document is to generate a consistent Agency approach for evaluating data on the chronic and subchronic effects of chemical mixtures. It is a procedural guide that emphasizes broad underlying principles of the various science disciplines (toxicology, pharmacology, statistics) necessary for assessing health risk from chemical mixture exposure. Approaches to be used with respect to the analysis and evaluation of the various data are also discussed.

It is not the intent of these Guidelines to regulate any social or economic aspects concerning risk of injury to human health or the environment caused by exposure to a chemical agent(s). All such action is addressed in specific statutes and federal legislation and is independent of these Guidelines.

While some potential environmental hazards involve significant exposure to only a single compound, most instances of environmental contamination involve concurrent or sequential exposures to a mixture of compounds that may induce similar or dissimilar effects over exposure periods ranging from short-term to lifetime. For the purposes of these Guidelines, mixtures will be defined as any combination of two or more chemical substances regardless of source or of spatial or temporal proximity. In some instances, the mixtures are highly complex, consisting of scores of compounds that are generated simultaneously as byproducts from a single source or process (e.g., coke oven emissions and diesel exhaust). In other cases, complex mixtures of related compounds are produced as commercial products (e.g., PCBs, gasoline and pesticide formulations) and eventually released to the environment. Another class of mixtures consists of compounds, often unrelated chemically or commercially, which are placed in the same area for disposal or storage, eventually come into contact with each other, and are released as a mixture to the environment. The quality and quantity of pertinent information available for risk assessment varies considerably for different mixtures. Occasionally, the chemical composition of a mixture is well characterized, levels of exposure to the population are known, and detailed toxicologic data on the mixture are available. Most frequently, not all components of the mixture are known, exposure data are uncertain, and toxicologic data on the known components of the mixture are limited. Nonetheless, the Agency may be required to take action because of the number of individuals at potential risk or because of the known toxicologic effects of these compounds that have been identified in the mixture.

The prediction of how specific mixtures of toxicants will interact must be based on an understanding of the mechanisms of such interactions. Most reviews and texts that discuss toxicant interactions attempt to discuss the biological or chemical bases of the interactions (e.g., Klaassen and Doull, 1980; Levine, 1973; Goldstein et al., 1974; NRC, 1980a; Veldstra, 1956; Withey, 1981). Although different authors use somewhat different classification schemes when discussing the ways in which toxicants interact, it generally is recognized that toxicant interactions may occur during any of the toxicologic processes that take place with a single compound: absorption, distribution, metabolism, excretion, and activity at the receptor site(s). Compounds may interact chemically, yielding a new toxic component or causing a change in the biological availability of the existing component. They may also interact by causing different effects at different receptor sites.

Because of the uncertainties inherent in predicting the magnitude and nature of toxicant interactions, the assessment of health risk from chemical mixtures must include a thorough discussion of all assumptions. No single approach is recommended in these Guidelines. Instead, guidance is given for the use of several approaches depending on the nature and quality of the data. Additional mathematical details are presented in Section 4.

In addition to these Guidelines, a supplemental technical support document is being developed which will contain a thorough review of all available information on the toxicity of chemical mixtures and a discussion of research needs.

2. PROPOSED APPROACH

No single approach can be recommended to risk assessments for multiple chemical exposures. Nonetheless, general guidelines can be recommended depending on the type of mixture, the known toxic effects of its components, the availability of toxicity data on the mixture or similar mixtures, the known or anticipated interactions among components of the mixture, and the quality of the exposure data. Given the complexity of this issue and the relative paucity of empirical data from which sound generalizations can be constructed, emphasis must be placed on flexibility, judgment, and a clear articulation of the assumptions and limitations in any risk assessment that is developed. The proposed approach is summarized in Table 1 and Figure 1 and is detailed below. An alphanumeric scheme for ranking the quality of the data used in the risk assessment is given in Table 2.

2.1. DATA AVAILABLE ON THE MIXTURE OF CONCERN

For predicting the effects of subchronic or chronic exposure to mixtures, the preferred approach usually will be to use subchronic or chronic health effects data on the mixture of

Table 1. Risk assessment approach for chemical mixtures

1. Assess the quality of the data on interactions, health effects, and exposure (see Table 2).
   a. If adequate, proceed to Step 2.
   b. If inadequate, proceed to Step 14.
2. Health effects information is available on the chemical mixture of concern.
   a. If yes, proceed to Step 3.
   b. If no, proceed to Step 4.
3. Conduct risk assessment on the mixture of concern based on health effects data on the mixture. Use the same procedures as those for single compounds. Proceed to Step 7 (optional) and Step 12.
4. Health effects information is available on a mixture that is similar to the mixture of concern.
   a. If yes, proceed to Step 5.
   b. If no, proceed to Step 7.
5. Assess the similarity of the mixture on which health effects data are available to the mixture of concern, with emphasis on any differences in components or proportions of components, as well as the effects that such differences would have on biological activity.
   a. If sufficiently similar, proceed to Step 6.
   b. If not sufficiently similar, proceed to Step 7.
6. Conduct risk assessment on the mixture of concern based on health effects data on the similar mixture. Use the same procedures as those for single compounds. Proceed to Step 7 (optional) and Step 12.
7. Compile health effects and exposure information on the components of the mixture.
8. Derive appropriate indices of acceptable exposure and/or risk on the individual components in the mixture. Proceed to Step 9.
9. Assess data on interactions of components in the mixtures.
   a. If sufficient quantitative data are available on the interactions of two or more components in the mixture, proceed to Step 10.
   b. If sufficient quantitative data are not available, use whatever information is available to qualitatively indicate the nature of potential interactions. Proceed to Step 11.
10. Use an appropriate interaction model to combine risk assessments on compounds for which data are adequate, and use an additivity assumption for the remaining compounds. Proceed to Step 11 (optional) and Step 12.
11. Develop a risk assessment based on an additivity approach for all compounds in the mixture. Proceed to Step 12.
12. Compare risk assessments conducted in Steps 5, 8, and 9. Identify and justify the preferred assessment, and quantify uncertainty, if possible. Proceed to Step 13.
13. Develop an integrated summary of the qualitative and quantitative assessments with special emphasis on uncertainties and assumptions. Classify the overall quality of the risk assessment, as indicated in Table 2. Stop.
14. No risk assessment can be conducted because of inadequate data on interactions, health effects, or exposure. Qualitatively assess the nature of any potential hazard and detail the types of additional data necessary to support a risk assessment. Stop.

Note—Several decisions used here, especially those concerning adequacy of data and similarity between two mixtures, are not precisely characterized and will require considerable judgment. See text.

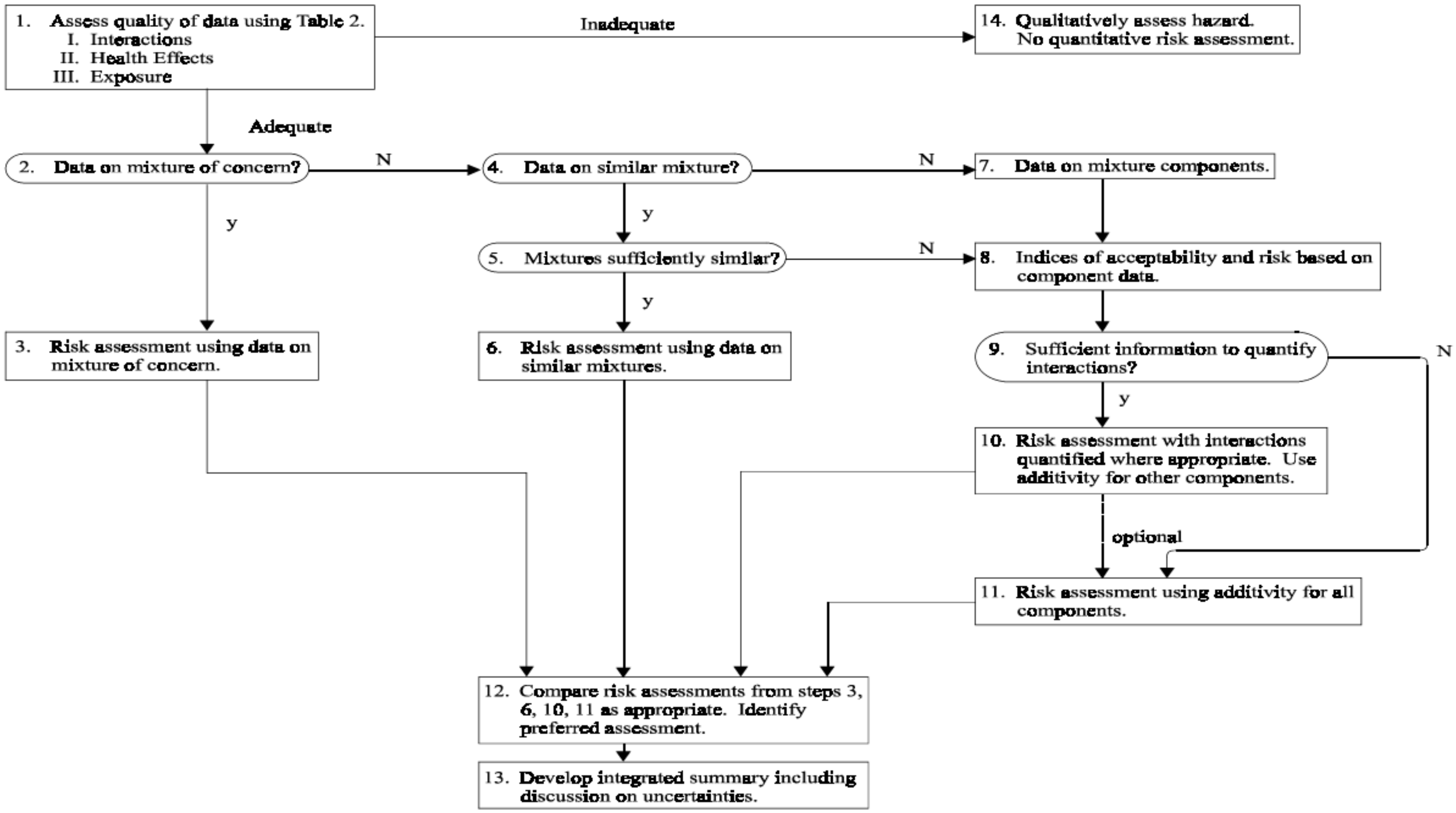

Figure 1. Flow chart of the risk assessment in Table 1. Note that it may be desirable to conduct all three assessments when possible (i.e., using data on the mixture, a similar mixture, or the components) in order to make the fullest use of the available data. See text for further discussion.

Table 2. Classification scheme for the quality of the risk assessment of the mixture[a]

Information on Interactions

I. Assessment is based on data on the mixture of concern.
II. Assessment is based on data on a sufficiently similar mixture.
III. Quantitative interactions of components are well characterized.
IV. The assumption of additivity is justified based on the nature of the health effects and on the number of component compounds.
V. An assumption of additivity cannot be justified, and no quantitative risk assessment can be conducted.

Health Effects Information

A. Full health effects data are available and relatively minor extrapolation is required.
B. Full health effects data are available but extensive extrapolation is required for route or duration of exposure or for species differences. These extrapolations are supported by pharmacokinetic considerations, empirical observations, or other relevant information.
C. Full health effects data are available, but extensive extrapolation is required for route or duration of exposure or for species differences. These extrapolations are not directly supported by the information available.
D. Certain important health effects data are lacking and extensive extrapolations are required for route or duration of exposure or for species differences.
E. A lack of health effects information on the mixture and its components in the mixture precludes a quantitative risk assessment.

Exposure Information[b]

1. Monitoring information either alone or in combination with modeling information is sufficient to accurately characterize human exposure to the mixture or its components.
2. Modeling information is sufficient to reasonably characterize human exposure to the mixture or its components.
3. Exposure estimates for some components are lacking, uncertain, or variable. Information on health effects or environmental chemistry suggests that this limitation is not likely to substantially affect the risk assessment.
4. Not all components in the mixture have been identified, or levels of exposure are highly uncertain or variable. Information on health effects or environmental chemistry is not sufficient to assess the effect of this limitation on the risk assessment.
5. The available exposure information is insufficient for conducting a risk assessment.

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[a]See text for discussion of sufficient similarity, adequacy of data, and justification for additivity assumptions.
[b]See the Agency's Guidelines for Estimating Exposures (U.S. EPA, 1986d) for more complete information on performing exposure assessments and evaluating the quality of exposure data.

concern and adopt procedures similar to those used for single compounds, either systemic toxicants or carcinogens (see U.S. EPA, 1986a-c). The risk assessor must recognize, however, that dose-response models used for single compounds are often based on biological mechanisms of the toxicity of single compounds, and may not be as well justified when applied to the mixture as a whole. Such data are most likely to be available on highly complex mixtures, such as coke oven emissions or diesel exhaust, which are generated in large quantities and associated with or suspected of causing adverse health effects. Attention should also be given to the persistence of the mixture in the environment as well as to the variability of the mixture composition over time or from different sources of emissions. If the components of the mixture are known to partition into different environmental compartments or to degrade or transform at different rates in the environment, then those factors must also be taken into account, or the confidence in and applicability of the risk assessment are diminished.

2.2. DATA AVAILABLE ON SIMILAR MIXTURES

If the risk assessment is based on data from a single mixture that is known to be generated with varying compositions depending on time or different emission sources, then the confidence in the applicability of the data to a risk assessment also is diminished. This can be offset to some degree if data are available on several mixtures of the same components that have different component ratios which encompass the temporal or spatial differences in composition of the mixture of concern. If such data are available, an attempt should be made to determine if significant and systematic differences exist among the chemical mixtures. If significant differences are noted, ranges of risk can be estimated based on the toxicologic data of the various mixtures. If no significant differences are noted, then a single risk assessment may be adequate, although the range of ratios of the components in the mixtures to which the risk assessment applies should also be given.

If no data are available on the mixtures of concern, but health effects data are available an a similar mixture (i.e., a mixture having the same components but in slightly different ratios, or having several common components but lacking one or more components, or having one or more additional components), a decision must be made whether the mixture on which health effects data are available is or is not "sufficiently similar" to the mixture of concern to permit a risk assessment. The determination of "sufficient similarity" must be made on a case-by-case basis, considering not only the uncertainties associated with using data on a dissimilar mixture but also the uncertainties of using other approaches such as additivity. In determining reasonable similarity, consideration should be given to any information on the components that differ or are contained in markedly different proportions between the mixture on which health effects data are available and the mixture of concern. Particular emphasis should be placed on any toxicologic or

pharmacokinetic data on the components or the mixtures which would be useful in assessing the significance of any chemical difference between the similar mixture and the mixtures of concern.

Even if a risk assessment can be made using data on the mixtures of concern or a reasonably similar mixture, it may be desirable to conduct a risk assessment based on toxicity data on the components in the mixture using the procedure outlined in Section 2.B. In the case of a mixture containing carcinogens and toxicants, an approach based on the mixture data alone may not be sufficiently protective in all cases. For example, this approach for a two-component mixture of one carcinogen and one toxicant would use toxicity data on the mixture of the two compounds. However, in a chronic study of such a mixture, the presence of the toxicant could mask the activity of the carcinogen. That is to say, at doses of the mixture sufficient to induce a carcinogenic effect, the toxicant could induce mortality so that at the maximum tolerated dose of the mixture, no carcinogenic effect could be observed. Since carcinogenicity is considered by the Agency to be a nonthreshold effect, it may not be prudent to construe the negative results of such a bioassay as indicating the absence of risk at lower doses. Consequently, the mixture approach should be modified to allow the risk assessor to evaluate the potential for masking, of one effect by another, on a case-by-case basis.

2.3. DATA AVAILABLE ONLY ON MIXTURE COMPONENTS

If data are not available on an identical or reasonably similar mixture, the risk assessment may be based on the toxic or carcinogenic properties of the components in the mixture. When little or no quantitative information is available on the potential interaction among the components, additive models (defined in the next section) are recommended for systemic toxicants. Several studies have demonstrated that dose additive models often predict reasonably well the toxicities of mixtures composed of a substantial variety of both similar and dissimilar compounds (Pozzani et al., 1959; Smyth et al., 1969, 1970; Murphy, 1980). The problem of multiple toxicant exposure has been addressed by the American Conference of Governmental Industrial Hygienists (ACGIH, 1983), the Occupational Safety and Health Administration (OSHA, 1983), the World Health Organization (WHO, 1981), and the National Research Council (NRC, 1980a,b). Although the focus and purpose of each group was somewhat different, all groups that recommended an approach elected to adopt some type of dose additive model. Nonetheless, as discussed in Section 4, dose additive models are not the most biologically plausible approach if the compounds do not have the same mode of toxicologic action. Consequently, depending on the nature of the risk assessment and the available information on modes of action and patterns of joint action, the Federal Register most reasonable additive model should be used.

2.3.1. Systemic Toxicants

For systemic toxicants, the current risk assessment methodology used by the Agency for single compounds most often results in the derivation of an exposure level which is not anticipated to cause significant adverse effects. Depending on the route of exposure, media of concern, and the legislative mandate guiding the risk assessments, these exposure levels may be expressed in a variety of ways such as acceptable daily intakes (ADIs) or reference doses (RfDs), levels associated with various margins of safety (MOS), or acceptable concentrations in various media. For the purpose of this discussion, the term "acceptable level" (AL) will be used to indicate any such criteria or advisories derived by the Agency. Levels of exposure (E) will be estimates obtained following the most current Agency Guidelines for Estimating Exposures (U.S. EPA, 1986d). For such estimates, the "hazard index" (HI) of a mixture based on the assumption of dose addition may be defined as:

$$HI = E_1/AL_1 + E_2/AL_2 + \ldots + E_i/AL_i \qquad (2\text{-}1)$$

where:

E_i = exposure level to the i^{th} toxicant* and AL_i = maximum acceptable level for the i^{th} toxicant.

Since the assumption of dose addition is most properly applied to compounds that induce the same effect by similar modes of action, a separate hazard index should be generated for each end point of concern. Dose addition for dissimilar effects does not have strong scientific support, and, if done, should be justified on a case-by-case basis in terms of biological plausibility.

The assumption of dose addition is most clearly justified when the mechanisms of action of the compounds under consideration are known to be the same. Since the mechanisms of action for most compounds are not well understood, the justification of the assumption of dose addition will often be limited to similarities in pharmacokinetic and toxicologic characteristics. In any event, if a hazard index is generated the quality of the experimental evidence supporting the assumption of dose addition must be clearly articulated.

The hazard index provides a rough measure of likely toxicity and requires cautious interpretation. The hazard index is only a numerical indication of the nearness to acceptable limits of exposure or the degree to which acceptable exposure levels are exceeded. As this index approaches unity, concern for the potential hazard of the mixture increases. If the index exceeds unity, the concern is the same as if an individual chemical exposure exceeded its acceptable level by the same proportion. The hazard index does not define dose-response relationships, and its numerical value should not be construed to be a direct estimate of risk. Nonetheless, if sufficient

data are available to derive individual acceptable levels for a spectrum of effects (e.g., MFO induction, minimal effects in several organs, reproductive effects, and behavioral effects), the hazard index may suggest what types of effects might be expected from the mixture exposure. If the components' variabilities of the acceptable levels are known, or if the acceptable levels are given as ranges (e.g., associated with different margins of safety), then the hazard index should be presented with corresponding estimates of variation or range.

Most studies on systemic toxicity report only descriptions of the effects in each dose group. If dose-response curves are estimated for systemic toxicants, however, dose-additive or response-additive assumptions can be used, with preference given to the most biologically plausible assumption (see Section 4 for the mathematical details).

2.3.2. Carcinogens

For carcinogens, whenever linearity of the individual dose-response curves has been assumed (usually restricted to low doses), the increase in risk P (also called excess or incremental risk), caused by exposure d, is related to carcinogenic potency B, as:

$$P = d\,B \qquad (2\text{-}2)$$

For multiple compounds, this equation may be generalized to:

$$P = \Sigma\, d_i\, B_i \qquad (2\text{-}3)$$

This equation assumes independence of action by the several carcinogens and is equivalent to the assumption of dose addition as well as to response addition with completely negative correlation of tolerance, as long as $P < 1$ (see Section 4). Analogous to the procedure used in Equation 2-1 for systemic toxicants, an index for n carcinogens can be developed by dividing exposure levels (E) by doses (DR) associated with a set level of risk:

$$HI = E_1/DR_1 + E_2/DR_2 + \ldots + E_n/DR_n \qquad (2\text{-}4)$$

Note that the less linear the dose-response curve is, the less appropriate Equations 2-3 and 2-4 will be, perhaps even at low doses. It should be emphasized that because of the uncertainties in estimating dose-response relationships for single compounds, and the additional uncertainties in combining the individual estimate to assess response from exposure to mixtures, response rates and hazard indices may have merit in comparing risks but should not be regarded as measures of absolute risk.

2.3.3. Interactions

None of the above equations incorporates any form of synergistic or antagonistic interaction. Some types of information, however, may be available that suggest that two or more components in the mixture may interact. Such information must be assessed in terms of both its relevance to subchronic or chronic hazard and its suitability for quantitatively altering the risk assessment.

For example, if chronic or subchronic toxicity or carcinogenicity studies have been conducted that permit a quantitative estimation of interaction for two chemicals, then it may be desirable to consider using equations detailed in Section 4, or modifications of these equations, to treat the two compounds as a single toxicant with greater or lesser potency than would be predicted from additivity. Other components of the mixture, on which no such interaction data are available, could then be separately treated in an additive manner. Before such a procedure is adopted, however, a discussion should be presented of the likelihood that other compounds in the mixture may interfere with the interaction of the two toxicants on which quantitative interaction data are available. If the weight of evidence suggests that interference is likely, then a quantitative alteration of the risk assessment may not be justified. In such cases, the risk assessment may only indicate the likely nature of interactions, either synergistic or antagonistic, and not quantify their magnitudes.

Other types of information, such as those relating to mechanisms of toxicant interaction, or quantitative estimates of interaction between two chemicals derived from acute studies, are even less likely to be of use in the quantitative assessment of long-term health risks. Usually it will be appropriate only to discuss these types of information, indicate the relevance of the information to subchronic or chronic exposure, and indicate, if possible, the nature of potential interactions, without attempting to quantify their magnitudes.

When the interactions are expected to have a minor influence on the mixture's toxicity, the assessment should indicate, when possible, the compounds most responsible for the predicted toxicity. This judgment should be based on predicted toxicity of each component, based on exposure and toxic or carcinogenic potential. This potential alone should not be used as an indicator of the chemicals posing the most hazard.

2.3.4. Uncertainties

For each risk assessment, the uncertainties should be clearly discussed and the overall quality of the risk assessment should be characterized. The scheme outlined in Table 2 should be used to express the degree of confidence in the quality of the data on interaction, health effects, and exposure.

a. Health Effects—In some cases, when health effects data are incomplete, it may be possible to argue by analogy or quantitative structure-activity relationships that the compounds on which no health effects data are available are not likely to significantly affect the toxicity of the mixture. If a risk assessment includes such an argument, the limitations of the approach must be clearly articulated. Since a methodology has not been adopted for estimating an acceptable level (e.g., ADI) or carcinogenic potential for single compounds based either on quantitative structure-activity relationships or on the results of short-term screening tests, such methods are not at present recommended as the sole basis of a risk assessment on chemical mixtures.

b. Exposure Uncertainties—The general uncertainties in exposure assessment have been addressed in the Agency's Guidelines for Estimating Exposures (U.S. EPA, 1986d). The risk assessor should discuss these exposure uncertainties in terms of the strength of the evidence used to quantify the exposure. When appropriate, the assessor should also compare monitoring and modeling data and discuss any inconsistencies as a source of uncertainty. For mixtures, these uncertainties may be increased as the number of compounds of concern increases.

   If levels of exposure to certain compounds known to be in the mixture are not available, but information on health effects and environmental persistence and transport suggest that these compounds are not likely to be significant in affecting the toxicity of the mixture, then a risk assessment can be conducted based on the remaining compounds in the mixture, with appropriate caveats. If such an argument cannot be supported, no final risk assessment can be performed until adequate monitoring data are available. As an interim procedure, a risk assessment may be conducted for those components in the mixture for which adequate exposure and health effects data are available. If the interim risk assessment does not suggest a hazard, there is still concern about the risk from such a mixture because not all components in the mixture have been considered.

c. Uncertainties Regarding Composition of the Mixture—In perhaps a worst-case scenario, information may be lacking not only on health effects and levels of exposure, but also on the identity of some components of the mixture. Analogous to the procedure described in the previous paragraph, an interim risk assessment can be conducted on those components of the mixture for which adequate health effects and exposure information are available. If the risk is considered unacceptable, a conservative approach is to present the quantitative estimates of risk, along with appropriate qualifications regarding the incompleteness of the data. If no hazard is indicated by this partial assessment, the risk assessment should not be quantified until better health effects and monitoring data are available to adequately characterize the mixture exposure and potential hazards.

3. ASSUMPTIONS AND LIMITATIONS

3.1. INFORMATION ON INTERACTIONS

Most of the data available on toxicant interactions are derived from acute toxicity studies using experimental animals in which mixtures of two compounds were tested, often in only a single combination. Major areas of uncertainty with the use of such data involve the appropriateness of interaction data from an acute toxicity study for quantitatively altering a risk assessment for subchronic or chronic exposure, the appropriateness of interaction data on two component mixtures for quantitatively altering a risk assessment on a mixture of several compounds, and the accuracy of interaction data on experimental animals for quantitatively predicting interactions in humans.

The use of interaction data from acute toxicity studies to assess the potential interactions on chronic exposure is highly questionable unless the mechanisms of the interaction on acute exposure were known to apply to low-dose chronic exposure. Most known biological mechanisms for toxicant interactions, however, involve some form of competition between the chemicals or phenomena involving saturation of a receptor site or metabolic pathway. As the doses of the toxicants are decreased, it is likely that these mechanisms either no longer will exert a significant effect or will be decreased to an extent that cannot be measured or approximated.

The use of information from two-component mixtures to assess the interactions in a mixture containing more than two compounds also is questionable from a mechanistic perspective. For example, if two compounds are known to interact, either synergistically or antagonistically, because of the effects of one compound on the metabolism or excretion of the other, the addition of a third compound which either chemically alters or affects the absorption of one of the first two compounds could substantially alter the degree of the toxicologic interaction. Usually, detailed studies quantifying toxicant interactions are not available on multicomponent mixtures, and the few studies that are available on such mixtures (e.g., Gullino et al., 1956) do not provide sufficient information to assess the effects of interactive interference. Concerns with the use of interaction data on experimental mammals to assess interactions in humans is based on the increasing appreciation for systematic differences among species in their response to individual chemicals. If systematic differences in toxic sensitivity to single chemicals exist among species, then it seems reasonable to suggest that the magnitude of toxicant interactions among species also may vary in a systematic manner.

Consequently, even if excellent chronic data are available on the magnitude of toxicant interactions in a species of experimental mammal, there is uncertainty that the magnitude of the interaction will be the same in humans. Again, data are not available to properly assess the significance of this uncertainty.

Last, it should be emphasized that none of the models for toxicant interaction can predict the magnitude of toxicant interactions in the absence of extensive data. If sufficient data are available to estimate interaction coefficients as described in Section 4, then the magnitude of the toxicant interactions for various proportions of the same components can be predicted. The availability of an interaction ratio (observed response divided by predicted response) is useful only in assessing the magnitude of the toxicant interaction for the specific proportions of the mixture which was used to generate the interaction ratio.

The basic assumption in the recommended approach is that risk assessments on chemical mixtures are best conducted using toxicologic data on the mixture of concern or a reasonably similar mixture. While such risk assessments do not formally consider toxicologic interactions as part of a mathematical model, it is assumed that responses in experimental mammals or human populations noted after exposure to the chemical mixture can be used to conduct risk assessments on human populations. In bioassays of chemical mixtures using experimental mammals, the same limitations inherent in species-to-species extrapolation for single compounds apply to mixtures. When using health effects data on chemical mixtures from studies on exposed human populations, the limitations of epidemiologic studies in the risk assessment of single compounds also apply to mixtures. Additional limitations may be involved when using health effects data on chemical mixtures if the components in the mixture are not constant or if the components partition in the environment.

3.2. ADDITIVITY MODELS

If sufficient data are not available on the effects of the chemical mixture of concern or a reasonably similar mixture, the proposed approach is to assume additivity. Dose additivity is based on the assumption that the components in the mixture have the same mode of action and elicit the same effects. This assumption will not hold true in most cases, at least for mixtures of systemic toxicants. For systemic toxicants, however, most single compound risk assessments will result in the derivation of acceptable levels, which, as currently defined, cannot be adapted to the different forms of response additivity as described in Section 4.

Additivity models can be modified to incorporate quantitative data on toxicant interactions from subchronic or chronic studies using the models given in Section 4 or modifications of these models. If this approach is taken, however, it will be under the assumption that other components in the mixture do not interfere with the measured interaction. In practice, such subchronic or chronic interactions data seldom will be available. Consequently, most risk assessments (on mixtures) will be based on an assumption of additivity, as long as the components elicit similar effects.

Dose-additive and response-additive assumptions can lead to substantial errors in risk estimates if synergistic or antagonistic interactions occur. Although dose additivity has been shown to predict the acute toxicities of many mixtures of similar and dissimilar compounds (e.g., Pozzani et al., 1959; Smyth et al., 1969, 1970; Murphy, 1980), some marked exceptions have been noted. For example, Smyth et al. (1970) tested the interaction of 53 pairs of industrial chemicals based on acute lethality in rats. For most pairs of compounds, the ratio of the predicted LD_{50} to observed LD_{50} did not vary by more than a factor of 2. The greatest variation was seen with an equivolume mixture of morpholine and toluene, in which the observed LD_{50} was about five times less than the LD_{50} predicted by dose addition. In a study by Hammond et al. (1979), the relative risk of lung cancer attributable to smoking was 11, while the relative risk associated with asbestos exposure was 5. The relative risk of lung cancer from both smoking and asbestos exposure was 53, indicating a substantial synergistic effect. Consequently, in some cases, additivity assumptions may substantially underestimate risk. In other cases, risk may be overestimated. While this is certainly an unsatisfactory situation, the available data on mixtures are insufficient for estimating the magnitude of these errors. Based on current information, additivity assumptions are expected to yield generally neutral risk estimates (i.e., neither conservative nor lenient) and are plausible for component compounds that induce similar types of effects at the same sites of action.

4. MATHEMATICAL MODELS AND THE MEASUREMENT OF JOINT ACTION

The simplest mathematical models for joint action assume no interaction in any mathematical sense. They describe either dose addition or response addition and are motivated by data on acute lethal effects of mixtures of two compounds.

4.1. DOSE ADDITION

Dose addition assumes that the toxicants in a mixture behave as if they were dilutions or concentrations of each other, thus the true slopes of the dose-response curves for the individual compounds are identical, and the response elicited by the mixture can be predicted by summing the individual doses after adjusting for differences in potency; this is defined as the ratio of equitoxic doses. Probit transformation typically makes this ratio constant at all doses when parallel straight lines are obtained. Although this assumption can be applied to any model (e.g., the one-hit model in NRC, 1980b), it has been most often used in toxicology with the log-dose probit response model, which will be used to illustrate the assumption of dose addition. Suppose that two toxicants show the following log-dose probit response equations:

$$Y_1 = 0.3 + 3 \log Z_1 \qquad (4\text{-}1)$$

$$Y_2 = 1.2 + 3 \log Z_2 \qquad (4\text{-}2)$$

where Y_1 is the probit response associated with a dose of Z_1 (i = 1, 2). The potency, p, of toxicant #2 with respect to toxicant #1 is defined by the quantity Z_1/Z_2 when $Y_1 = Y_2$ (that is what is meant by equitoxic doses). In this example, the potency, p, is approximately 2. Dose addition assumes that the response, Y, to any mixture of these two toxicants can be predicted by

$$Y = 0.3 + 3 \log (Z_1 + pZ_2) \qquad (4\text{-}3)$$

Thus, since p is defined as Z_1/Z_2, Equation 4-3 essentially converts Z_2 into an equivalent dose of Z_1 by adjusting for the difference in potency. A more generalized form of this equation for any number of toxicants is:

$$Y = a_1 + b \log (f_1 + \Sigma\, f_i p_i) + b \log Z \qquad (4\text{-}4)$$

where:

a_1 = the y-intercept of the dose-response equation for toxicant #1
b = the slope of the dose-response lines for the toxicants
f_i = the proportion of the i^{th} toxicant in the mixture
p_i = the potency of the i^{th} toxicant with respect to toxicant #1 (i.e., Z_1/Z_i); and
Z = the sum of the individual doses in the mixture.

A more detailed discussion of the derivation of the equations for dose addition is presented by Finney (1971).

4.2. RESPONSE ADDITION

The other form of additivity is referred to as response addition. As detailed by Bliss (1939), this type of joint action assumes that the two toxicants act on different receptor systems and that the correlation of individual tolerances may range from completely negative (r = –1) to completely positive (r = + 1). Response addition assumes that the response to a given concentration of a mixture of toxicants is completely determined by the responses to the components and the pairwise correlation coefficient. Taking P as the proportion of organisms responding to a mixture of two toxicants which evoke individual responses of P_1 and P_2, then.

$P = P_1$ if $r = 1$ and $P_1 \geq P_2$ (4-5)

$P = P_2$ if $r = 1$ and $P_1 < P_2$ (4-6)

$P = P_1 + P_2\,(1 - P_1)$ if $r = 0$ (4-7)

$P = P_1 + P_2$ if $r = -1$ and $P \leq 1$. (4-8)

More generalized mathematical models for this form of joint action have been given by Plackett and Hewlett (1948).

4.3. INTERACTIONS

All of the above models assume no interactions and therefore do not incorporate measurements of synergistic or antagonistic effects. For measuring toxicant interactions for mixtures of two compounds, Finney (1942) proposed the following modification of Equation 4-4 for dose addition:

$$Y = a_1 + b \log (f_1 + pf_2 + K\,[pf_1f_2]^{0\ 5}) + b \log Z \qquad (4\text{-}9)$$

where a_1, b, f_1, f_2, p, and Z are defined as before, and K is the coefficient of interaction. A positive value of K indicates synergism, a negative value indicates antagonism, and a value of zero corresponds to dose addition as in Equation 4-4. Like other proposed modifications of dose addition (Hewlett, 1969), the equation assumes a consistent interaction throughout the entire range of proportions of individual components. To account for such asymmetric patterns of interaction as those observed by Alstott et al. (1973), Durkin (1981) proposed the following modification to Equation 4-9:

$$Y = a_1 + b \log (f_1 + pf_2 + K_1f_1\,[pf_1f_2]^{0\ 5} + K_2f_2[pf_1f_2]^{0\ 5}) + b \log z \qquad (4\text{-}10)$$

in which $K(pf_1f_2)^{0\ 5}$ is divided into two components, $K_1f_1\,(pf_1f_2)^{0\ 5}$ and $K_2f_2[pf_1f_2]^{0\ 5}$. Since K_1 and K_2 need not have the same sign, apparent instances of antagonism at one receptor site and synergism at another receptor site can be estimated. When K_1 and K_2 are equal, Equation 4-10 reduces to Equation 4-9.

It should be noted that to obtain a reasonable number of degrees of freedom in the estimation of K in Equation 4-9 or K_1 and K_2 in Equation 4-10, the toxicity of several different combinations of the two components must be assayed along with assays of the toxicity of the individual components. Since this requires experiments with large numbers of animals, such analyses have been restricted for the most part to data from acute bioassays using insects (e.g., Finney, 1971) or aquatic organisms (Durkin, 1979). Also, because of the complexity of

experimental design and the need for large numbers of animals, neither Equation 4-9 nor Equation 4-10 has been generalized or applied to mixtures of more than two toxicants. Modifications of response-additive models to include interactive terms have also been proposed, along with appropriate statistical tests for the assumption of additivity (Korn and Liu, 1983; Wahrendorf et al., 1981).

In the epidemiologic literature, measurements of the extent of toxicant interactions, S, can be expressed as the ratio of observed relative risk to relative risk predicted by some form of additivity assumption. Analogous to the ratio of interaction in classical toxicology studies, S = 1 indicates no interaction, S > 1 indicates synergism, and S < l indicates antagonism. Several models for both additive and multiplicative risks have been proposed (e.g., Hogan et al., 1978; NRC, 1980b; Walter, 1976). For instance, Rothman (1976) has discussed the use of the following measurement of toxicant interaction based on the assumption of risk additivity:

$$S = (R_{11} - 1)/(R_{10} + R_{01} - 2) \qquad (4\text{-}11)$$

where R_{10} is the relative risk from compound #1 in the absence of compound #2, R_{01} is the relative risk from compound #2 in the absence of compound #1, and R_{11} is the relative risk from exposure to both compounds. A multiplicative risk model adapted from Walter and Holford (1978, Equation 4) can be stated as:

$$S = R_{11}/(R_{10}\ R_{01}) \qquad (4\text{-}12)$$

As discussed by both Walter and Holford (1978) and Rothman (1976), the risk-additive model is generally applied to agents causing diseases while the multiplicative model is more appropriate to agents that prevent disease. The relative merits of these and other indices have been the subject of considerable discussion in the epidemiologic literature (Hogan et al., 1978; Kupper and Hogan, 1978; Rothman, 1978; Rothman et al., 1980; Walter and Holford, 1978). There seems to be a consensus that for public health concerns regarding causative (toxic) agents, the additive model is more appropriate.

Both the additive and multiplicative models assume statistical independence in that the risk associated with exposure to both compounds in combination can be predicted by the risks associated with separate exposure to the individual compounds. As illustrated by Siemiatycki and Thomas (1981) for multistage carcinogenesis, the better fitting statistical model will depend not only upon actual biological interactions, but also upon the stages of the disease process which the compounds affect. Consequently, there is no a priori basis for selecting either type of model in a risk assessment. As discussed by Stara et al. (1983), the concepts of

multistage carcinogenesis and the effects of promoters and cocarcinogens on risk are extremely complex issues. Although risk models for promoters have been proposed (e.g., Bums et al., 1983), no single approach can be recommended at this time.

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PART B: RESPONSE TO PUBLIC AND SCIENCE ADVISORY BOARD COMMENTS

1. INTRODUCTION

This section summarizes some of the major issues raised in public comments on the Proposed Guidelines for the Health Risk Assessment of Chemical Mixtures published on January 9, 1985 (50 FR 1170). Comments were received from 14 individuals or organizations. An issue paper reflecting public and external review comments was presented to the Chemical Mixtures Guidelines Panel of the Science Advisory Board (SAB) on March 4, 1985. At its April 22-23, 1985, meeting, the SAB Panel provided the Agency with additional suggestions and recommendations concerning the Guidelines. This section also summarizes the issues raised by the SAB.

The SAB and public commentators expressed diverse opinions and addressed issues from a variety of perspectives. In response to comments, the Agency has modified or clarified many sections of the Guidelines, and is planning to develop a technical support document in line with the SAB recommendations. The discussion that follows highlights significant issues raised in the comments, and the Agency's response to them. Also, many minor recommendations, which do not warrant discussion here, were adopted by the Agency.

2. RECOMMENDED PROCEDURES

2.1. DEFINITIONS

Several comments were received concerning the lack of definitions for certain key items and the general understandability of certain sections. Definitions have been rewritten for several terms and the text has been significantly rewritten to clarify the Agency's intent and meaning.

Several commentators noted the lack of a precise definition of "mixture," even though several classes of mixtures are discussed. In the field of chemistry, the term "mixture" is usually differentiated from true solutions, with the former defined as nonhomogeneous multicomponent systems. For these Guidelines, the term "mixture" is defined as ". . any combination of two or more chemicals regardless of spatial or temporal homogeneity of source" (Section 1). These Guidelines are intended to cover risk assessments for any situation where the population is exposed or potentially exposed to two or more compounds of concern. Consequently, the introduction has been revised to clarify the intended breadth of application.

Several commentators expressed concern that "sufficient similarity" was difficult to define and that the Guidelines should give more details concerning similar mixtures. The Agency agrees and is planning research projects to improve on the definition. Characteristics such as

composition and toxic end-effects are certainly important, but the best indicators of similarity in terms of risk assessment have yet to be determined. The discussion in the Guidelines emphasizes case-by-case judgment until the necessary research can be performed. The Agency considered but rejected adding an example, because it is not likely that any single example would be adequate to illustrate the variety in the data and types of judgments that will be required in applying this concept. Inclusion of examples is being considered for the technical support document.

2.2. MIXTURES OF CARCINOGENS AND SYSTEMIC TOXICANTS

The applicability of the preferred approach for a mixture of carcinogens and systemic (noncarcinogenic) toxicants was a concern of several public commentators as well as the SAB. The Agency realizes that the preferred approach of using test data on the mixture itself may not be sufficiently protective in all cases. For example, take a simple two-component mixture of one carcinogen and one toxicant. The preferred approach would lead to using toxicity data on the mixture of the two compounds. However, it is possible to set the proportions of each component so that in a chronic bioassay of such a mixture, the presence of the toxicant could mask the activity of the carcinogen. That is to say, at doses of the mixture sufficient for the carcinogen to induce tumors in the small experimental group, the toxicant could induce mortality. At a lower dose in the same study, no adverse effects would be observed, including no carcinogenic effects. The data would then suggest use of a threshold approach. Since carcinogenicity is considered by the Agency to be a nonthreshold effect, it may not be prudent to construe the negative results of such a bioassay as indicating the absence of risk at lower doses. Consequently, the Agency has revised the discussion of the preferred approach to allow the risk assessor to evaluate the potential for masking of carcinogenicity or other effects on a case-by-case basis.

Another difficulty occurs with such a mixture when the risk assessment needs to be based on data for the mixture components. Carcinogens and systemic toxicants are evaluated by the Agency using different approaches and generally are described by different types of data: response rates for carcinogens vs. effect descriptions for toxicants. The Agency recognizes this difficulty and recommends research to develop a new assessment model for combining these dissimilar data sets into one risk estimate. One suggestion in the interim is to present separate risk estimates for the dissimilar end points, including carcinogenic, teratogenic, mutagenic, and systemic toxicant components.

3. ADDITIVITY ASSUMPTION

Numerous comments were received concerning the assumption of additivity, including:

a. the applicability of additivity to "complex" mixtures;
b. the use of dose additivity for compounds that induce different effects;
c. the interpretation of the Hazard Index; and
d. the use of interaction data.

Parts of the discussion in the proposed guidelines concerning the use of additivity assumptions were vague and have been revised in the final Guidelines to clarify the Agency's intent and position.

3.1. COMPLEX MIXTURES

The issue of the applicability of an assumption of additivity to complex mixtures containing tens or hundreds of components was raised in several of the public comments. The Agency and its reviewers agree that as the number of compounds in the mixture increases, an assumption of additivity will become less reliable in estimating risk. This is based on the fact that each component estimate of risk or an acceptable level is associated with some error and uncertainty. With current knowledge, the uncertainty will increase as the number of components increases. In any event, little experimental data are available to determine the general change in the error as the mixture contains more components. The Agency has decided that a limit to the number of components should not be set in these Guidelines. However, the Guidelines do explicitly state that as the number of compounds in the mixture increases, the uncertainty associated with the risk assessment is also likely to increase.

3.2. DOSE ADDITIVITY

Commentators were concerned about what appeared to be a recommendation of the use of dose additivity for compounds that induce different effects. The discussion following the dose additivity equation was clarified to indicate that the act of combining all compounds, even if they induce dissimilar effects, is a screening procedure and not the preferred procedure in developing a hazard index. The Guidelines were further clarified to state that dose (or response) additivity is theoretically sound, and therefore best applied for assessing mixtures of similar acting components that do not interact.

3.3. INTERPRETATION OF THE HAZARD INDEX

Several comments addressed the potential for misinterpretation of the hazard index, and some questioned its validity, suggesting that it mixes science and value judgments by using "acceptable" levels in the calculation. The Agency agrees with the possible confusion regarding its use and has revised the Guidelines for clarification. The hazard index is an easily derived restatement of dose additivity, and is, therefore, most accurate when used with mixture components that have similar toxic action. When used with components of unknown or dissimilar action, the hazard index is less accurate and should be interpreted only as a rough indication of concern. As with dose addition, the uncertainty associated with the hazard index increases as the number of components increases, so that it is less appropriate for evaluating the toxicity of complex mixtures.

3.4. USE OF INTERACTION DATA

A few commentators suggested that any interaction data should be used to quantitatively alter the risk assessment. The Agency disagrees. The current information on interactions is meager, with only a few studies comparing response to the mixture with that predicted by studies on components. Additional uncertainties include exposure variations due to changes in composition, mixture dose, and species differences in the extent of the interaction. The Agency is constructing an interaction data base in an attempt to answer some of these issues. Other comments concerned the use of different types of interaction data. The Guidelines restrict the use of interaction data to that obtained from whole animal bioassays of a duration appropriate to the risk assessment. Since such data are frequently lacking, at least for chronic or subchronic effects, the issue is whether to allow for the use of other information such as acute data, *in vitro* data, or structure-activity relationships to quantitatively alter the risk assessment, perhaps by use of a safety factor. The Agency believes that sufficient scientific upport does not exist for the use of such data in any but a qualitative discussion of possible synergistic or antagonistic effects.

4. UNCERTAINTIES AND THE SUFFICIENCY OF THE DATA BASE

In the last two paragraphs of Section II of the Guidelines, situations are discussed in which the risk assessor is presented with incomplete toxicity, monitoring, or exposure data. The SAB, as well as several public commentors, recommended that the "risk management" tone of this section be modified and that the option of the risk assessor to decline to conduct a risk assessment be made more explicit.

This is a difficult issue that must consider not only the quality of the available data for risk assessment, but also the needs of the Agency in risk management. Given the types of poor

data often available, the risk assessor may indicate that the risk assessment is based on limited information and thus contains no quantification of risk. Nonetheless, in any risk assessment, substantial uncertainties exist. It is the obligation of the risk assessor to provide an assessment, but also to ensure that all the assumptions and uncertainties are articulated clearly and quantified whenever possible.

The SAB articulated several other recommendations related to uncertainties, all of which have been followed in the revision of the Guidelines. One recommendation was that the summary procedure table also be presented as a flow chart so that all options are clearly displayed. The SAB further recommended the development of a system to express the level of confidence in the various steps of the risk assessment.

The Agency has revised the summary table to present four major options: risk assessment using data on the mixture itself, data on a similar mixture, data on the mixture's components, or declining to quantify the risk when the data are inadequate. A flow chart of this table has also been added to more clearly depict the various options and to suggest the combining of the several options to indicate the variability and uncertainties in the risk assessment.

To determine the adequacy of the data, the SAB also recommended the development of a system to express the level of confidence associated with various steps in the risk assessment process. The Agency has developed a rating scheme to describe data quality in three areas: interaction, health effects, and exposure. This classification provides a range of five levels of data quality for each of the three areas. Choosing the last level in any area results in declining to perform a quantitative risk assessment due to inadequate data. These last levels are described as follows:

> Interactions: An assumption of additivity cannot be justified, and no quantitative risk assessment can be conducted.
>
> Health effects: A lack of health effects information on the mixture and its components precludes a quantitative risk assessment.
>
> Exposure: The available exposure information is insufficient for conducting a risk assessment.

Several commentors, including the SAB, emphasized the importance of not losing these classifications and uncertainties farther along in the risk management process. The discussion of uncertainties has been expanded in the final Guidelines and includes the recommendation that a

discussion of uncertainties and assumptions be included at every step of the regulatory process that uses risk assessment.

Another SAB comment was that the Guidelines should include additional procedures for mixtures with more than one end point or effect. The Agency agrees that these are concerns and revised the Guidelines to emphasize these as additional uncertainties worthy of further research.

5. NEED FOR A TECHNICAL SUPPORT DOCUMENT

The third major SAB comment concerned the necessity for a separate technical support document for these Guidelines. The SAB pointed out that the scientific and technical background from which these Guidelines must draw their validity is so broad and varied that it cannot reasonably be synthesized within the framework of a brief set of guidelines. The Agency is developing a technical support document that will summarize the available information on health effects from chemical mixtures, and on interaction mechanisms, as well as identify and develop mathematical models and statistical techniques to support these Guidelines. This document will also identify critical gaps and research needs.

Several comments addressed the need for examples on the use of the Guidelines. The Agency has decided to include examples in the technical support document.

Another issue raised by the SAB concerned the identification of research needs. Because little emphasis has been placed on the toxicology of mixtures until recently, the information on mixtures is limited. The SAB pointed out that identifying research needs is critical to the risk assessment process, and the EPA should ensure that these needs are considered in the research planning process. The Agency will include a section in the technical support document that identifies research needs regarding both methodology and data.